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The phenology of cyanobacteria blooms and carbon cycling in eutrophic lake ecosystems

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The phenology of cyanobacteria blooms and carbon cycling in eutrophic lake ecosystems

by

Ana M. Morales-Williams

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Co-Majors: Environmental Science and Ecology and Evolutionary Biology

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2016

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Work hard, be kind, and amazing things will happen.

-Conan O'Brien

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ABSTRACT

Anthropogenic eutrophication is fundamentally changing the role of lakes in global carbon cycles. Without eutrophication, lakes function as net sources of CO₂ to the atmosphere via watershed inputs and degradation of terrestrial organic carbon. In eutrophic lakes, however, this process can be reversed due to sustained phytoplankton blooms and thus high primary producer demand for CO₂. Global increases in these blooms over the past two decades suggest the theoretical framework we currently use to explain phytoplankton community assembly may not fully account for the environmental stochasticity associated with climate change processes and anthropogenic landscape manipulation. As human populations increase, so does the proportion of our terrestrial landscape devoted to agriculture and urban areas, and thus the proportion of inland waters subject to eutrophication. Moving forward, it is critical to understand the response of lakes to anthropogenic disturbance, both to better predict harmful blooms and to evaluate the changing role of lakes in global carbon cycles.

My dissertation addressed three primary questions: 1. Can eutrophication render lakes net sinks of atmospheric CO₂?, 2. When CO₂ is depleted from surface water, what mechanisms sustain cyanobacteria bloom biomass?, and 3. Do blooms act to stabilize or destabilize aquatic primary producer communities? To address these questions, sixteen eutrophic lakes were chosen along orthogonal gradients of interannual variability in watershed hydrologic permeability and Cyanobacteria dominance. My work demonstrated that in these lakes, CO₂ and dissolved inorganic carbon (DIC) were derived primarily from internal lake processes, and never from heterotrophic degradation of terrestrial organic carbon. Stable isotopic analyses revealed that DIC came from heterotrophic recycling of autochthonous carbon, atmospheric uptake, or mineral

dissolution. Additionally, as production increased and CO₂ was depleted from surface waters below atmospheric equilibrium, Cyanobacteria blooms developed that shifted from diffusive uptake of bioavailable CO₂ to energetically costly active uptake of scarce CO₂ or HCO₃⁻. This mechanism creates a positive feedback loop, where high production is maintained under CO₂ depletion, allowing eutrophic lakes to act as net carbon sinks. Finally, I show that long term cyclic fluctuations in cyanobacteria biomass are a mechanism of instability in primary producer communities. These findings suggest that as growing human populations force more nutrient intensive agriculture, lakes will continue to shift to impacted, eutrophic conditions. These processes have the potential to alter the global CO₂ budget and cause shifts to harmful algae that can efficiently use non-CO₂ DIC.

CHAPTER I

INTRODUCTION

Human activity has irreversibly altered biogeochemical cycling and ecosystem processes at a global scale (Vitousek, 1994; Foley *et al.*, 2005). Three quarters of ice-free land is actively used by humans. Much of this terrestrial landscape is now devoted to agriculture and urban centers, resulting in widespread biodiversity loss and eutrophication of downstream aquatic ecosystems. These terrestrial processes alter community structure, nutrient cycling, carbon storage, and gas flux in and from freshwater lakes (Paerl & Huisman, 2009; Heathcote & Downing, 2011; Jones *et al.*, 2016). Continued anthropogenic pressure and eutrophication of lake ecosystems is expected to result in increased sediment carbon storage, methane emission, and export of dissolved organic carbon to coastal systems (Cole *et al.*, 2007; Tranvik *et al.*, 2009). Globally, small lakes, ponds, and impoundments are more abundant than large ones (Downing & Cole, 2006), and are active processors of carbon and nutrients (Downing, 2010). Because these small water bodies dominate agricultural and urban landscapes, understanding their response to anthropogenic pressure is critical to evaluating the role of lakes in global biogeochemical cycles.

Harmful phytoplankton blooms are a characteristic feature of impacted lakes, and have increased globally in frequency and intensity over the past decade. These blooms are maintained by the interaction of climate change processes (warming, storm events), land use modification, and nutrient enrichment (Rigosi *et al.*, 2014), though specific

mechanisms that trigger and maintain them remain poorly understood. While we expect a single species to dominate at high ambient nutrient concentrations, we see variable community structure and succession within eutrophic and hypereutrophic lakes that have similar ambient nutrient concentrations. This suggests the theoretical equilibrium framework we use to describe and predict phytoplankton community succession does not explain the phenology of bloom forming communities in these systems.

Cyanobacteria blooms resulting from anthropogenic eutrophication severely degrade lake ecosystems via toxin production, carbon accumulation, hypoxia, food web alteration, and biodiversity loss (Heisler *et al.*, 2008; Paerl *et al.*, 2011; Brooks *et al.*, 2016). The high rates of primary productivity sustained by bloom communities alter the balance of production and respiration, changing gas flux regimes (Balmer & Downing, 2011; Pacheco *et al.*, 2014; Jones *et al.*, 2016). Because lakes are generally considered sources of CO₂ to the atmosphere (Cole *et al.*, 1994; Sobek *et al.*, 2005) these trends have serious implications for the role of lakes in global carbon cycles. Anthropogenic eutrophication has the potential to shift inland waters from net sources of CO₂ to net sinks, which would help mitigate increasing atmospheric CO₂ concentrations, except that lake-sediment carbon accumulation fuels production of methane, a more potent greenhouse gas than CO₂.

My dissertation explores physiological, biogeochemical, and ecological mechanisms that interact to drive phytoplankton phenology and carbon processing in eutrophic lake ecosystems. In Chapter 2, I explore the cyanobacterial carbon concentrating mechanism as a competitive strategy by which cyanobacteria can maintain high primary productivity when ambient CO₂ is depleted from surface waters. Chapter 3

describes extreme temporal patterns of CO₂ influx and efflux and compares these rates to those reported globally. Chapter 4 investigates nitrite photodegradation, reactive oxygen production, and organic carbon quality as mechanisms maintaining high CO₂ efflux when primary production is also high. Finally, Chapter 5 describes synchronous fluctuations of cyanobacterial communities at multiple time scales as a driver of destabilization of ecosystem function in eutrophic lakes. Together, this work aims to demonstrate that eutrophication has fundamentally altered community assembly and biogeochemical processing in aquatic systems, from organismal to ecosystem scales.

CHAPTER II

CARBON CONCENTRATING MECHANISMS MAINTAIN BLOOM BIOMASS AND CO₂ DEPLETION IN EUTROPHIC LAKE ECOSYSTEMS

Abstract

Harmful phytoplankton blooms are increasing in frequency, intensity, and duration in aquatic ecosystems worldwide. In many eutrophic lakes, these high levels of primary productivity correspond to periods of CO₂ depletion in surface waters. Cyanobacteria and other groups of phytoplankton have the ability to actively transport bicarbonate (HCO₃⁻) across their cell membrane when CO₂ concentrations are limiting, possibly giving them a competitive advantage over algae not using carbon concentrating mechanisms (CCMs). To investigate whether CCMs can maintain phytoplankton bloom biomass under CO₂ depletion, we measured $\delta^{13}\text{C}$ signatures of dissolved inorganic carbon ($\delta^{13}\text{C}_{\text{DIC}}$) and phytoplankton particulate organic carbon ($\delta^{13}\text{C}_{\text{phyto}}$) in sixteen mesotrophic to hypereutrophic lakes during the ice-free season of 2012. We used mass balance relationships to determine the dominant inorganic carbon species used by phytoplankton under CO₂ stress. We found a significant positive relationship between phytoplankton biomass and phytoplankton $\delta^{13}\text{C}$ signatures, as well as a significant non-linear negative relationship between water column ρCO_2 and isotopic composition of phytoplankton, indicating a shift from diffusive uptake to active uptake by phytoplankton of CO₂ or HCO₃⁻ during blooms. Calculated photosynthetic fractionation factors indicated that this shift occurs

specifically when surface water CO₂ drops below atmospheric equilibrium. Our results indicate active HCO₃⁻ uptake via CCMs may be an important mechanism maintaining phytoplankton blooms when CO₂ is depleted. Further increases in anthropogenic pressure, eutrophication, and harmful cyanobacteria blooms are therefore expected to contribute to increased bicarbonate uptake to sustain primary production.

Introduction

Harmful cyanobacteria blooms (HCBs) resulting from anthropogenic eutrophication are among the greatest current threats to inland water ecosystems, altering carbon cycling and ecosystem function, impairing water quality, and endangering human health (Paerl *et al.*, 2011; Brooks *et al.*, 2016; Visser *et al.*, 2016). Forecasting models and macrosystem-scale analyses suggest the occurrence of HCBs is driven by the interactive effects of land use, nutrient inputs (nitrogen and phosphorus), climate, weather, and in-lake processes (Michalak *et al.*, 2013; Rigosi *et al.*, 2014; Anneville *et al.*, 2015; Persaud *et al.*, 2015). Mechanisms determining variability in timing and duration of these events in lakes, however, remain poorly understood (Brooks *et al.*, 2016), and it is unclear what the large scale feedbacks of sustained primary production are on lake carbon cycling by phytoplankton. While temperate lakes have generally been considered net sources of CO₂ to the atmosphere (Tranvik *et al.*, 2009), eutrophic systems can maintain both high levels of primary production and negligible concentrations of CO₂ in surface water (Gu *et al.*, 2010; Balmer & Downing, 2011; Laas *et al.*, 2012), possibly increasing the flow of dissolved inorganic C to organic C. Identifying drivers of the temporal variability of bloom formation and maintenance will contribute to a better understanding of carbon dynamics in lakes with high productivity.

Cyanobacteria have developed a suite of diverse strategies for obtaining and fixing carbon and nutrients at growth-limiting concentrations. In addition to fixing atmospheric nitrogen, they are able to maintain metabolic processes under severe CO₂ depletion by use of a carbon concentrating mechanism (CCM; Badger and Price 2003; Raven *et al.* 2008). CCMs are present in many groups of aquatic photoautotrophs including green algae (Spalding, 2008) and diatoms (Hopkinson *et al.*, 2016), as well as some higher plants. These mechanisms are thought to have evolved independently in eukaryotic algae and the cyanobacteria, corresponding to a large decrease in atmospheric CO₂ and doubling of O₂ approximately 400 million years BP (Badger & Price, 2003; Raven *et al.*, 2008). Compared to less efficient eukaryotic CCMs, many cyanobacteria can concentrate dissolved inorganic carbon (DIC) to intracellular levels 1000 times greater than ambient concentrations (Raven *et al.*, 2008).

The cyanobacterial CCM mechanism facilitates active transport of HCO₃⁻ across the plasma membrane, where it is accumulated in the cytosol, transferred to Rubisco-containing carboxysomes, and converted to CO₂ via carbonic anhydrases (Raven *et al.*, 2008). In freshwaters, cyanobacteria use form 1B Rubisco, which facilitates acclimation to inorganic carbon depletion via high cellular affinity for CO₂ and HCO₃⁻ (Raven *et al.*, 2008; Shih *et al.*, 2015; Raven & Beardall, 2016). In addition to inorganic carbon availability, cyanobacterial CCMs are triggered by photosynthetically active radiation (PAR) and nitrogen availability. Because CCMs are energetically costly (Raven & Beardall, 2016), decreased PAR lowers cellular affinity for inorganic carbon (Giordano *et al.*, 2005). Affinity increases with depletion of nitrate and iron, but decreases with depletion of NH₄⁺, and does not have a consistent response to phosphorus limitation (Raven *et al.*, 2008). CCM activation under carbon and nutrient stress

thus may confer a competitive advantage to cyanobacteria via efficient carbon fixation when CO₂ is low (Badger & Price, 2003; Price *et al.*, 2008).

Shifts to alternative carbon assimilation strategies result in measureable changes in isotopic fractionation. Stable isotopic signatures of phytoplankton are dependent both on the isotopic composition of their DIC source and the physiological mechanism used to acquire it. When phytoplankton, including cyanobacteria, use passive diffusion to take up ambient CO₂, photosynthetic fractionation resembles that of C₃ terrestrial plants (Yoshioka, 1997), resulting in typical mean $\delta^{13}\text{C}$ signatures between -27‰ to -30‰ (O’Leary, 1988; Erez *et al.*, 1998; Bade *et al.*, 2004). In cyanobacteria and other phytoplankton, carbon fixation can be equally limited by carboxylation and active inorganic carbon transport into the cell. Cyanobacteria that are actively taking up HCO₃⁻ can have elevated $\delta^{13}\text{C}$ values as high as -8 to -11‰ (Sharkey & Berry, 1985; Vuorio *et al.*, 2006). This is largely attributable to the isotopic signature of source material (Kaplan & Reinhold, 1999), as well as decreased carbon efflux when CCMs are active, resulting in reduced photosynthetic fractionation (-1‰ to -3‰; Sharkey and Berry 1985; Erez et al. 1998). Further, isotopic fractionation associated with active HCO₃⁻ uptake is negligible (Sharkey & Berry, 1985; Yoshioka, 1997). In other words, discrimination due to passive diffusion is reduced or negligible when active HCO₃⁻ uptake is occurring (Giordano *et al.*, 2005). Thus, if CCMs are activated during cyanobacteria blooms in eutrophic lakes, we would expect the $\delta^{13}\text{C}$ signature of the phytoplankton to increase as ambient CO₂ is depleted, and photosynthetic fractionation factors to decrease as the community approaches a monoculture of phytoplankton using CCM.

The purpose of this study was to evaluate the importance of CCMs in maintaining high phytoplankton biomass during CO₂ depletion in eutrophic and hypereutrophic lakes. We hypothesized that photosynthetic fractionation would be tightly coupled with inorganic carbon

limitation, resulting in decreased photosynthetic fractionation as dissolved CO₂ shifts to mineral HCO₃⁻ in the water column. We further hypothesized that phytoplankton isotopic composition and photosynthetic fractionation would correspond to water column CO₂ depletion, reflecting CCM activation during blooms.

Methods

16 lakes were chosen based on Iowa State Limnology Laboratory long-term survey data (total phosphorus and phytoplankton community composition, 2000-2010, data publically available at: <http://limnology.eeob.iastate.edu/lakereport/>) including lakes with flashy watersheds (Fraterrigo & Downing, 2008) and those with high and low interannual variability in cyanobacteria dominance. Long-term survey data were used only for site selection. Duplicate stable isotope samples for particulate organic and dissolved inorganic analyses were collected once following ice off in 2012, weekly May-July, bi-weekly in August, and monthly September-November ($n=196$). Standard physical, chemical, and biological parameters were measured at each sampling event using US-EPA certified methods, including total nitrogen (TN), total phosphorus (TP), chlorophyll a (Chl a), alkalinity, vertical depth profiles of temperature, pH, conductivity, and dissolved oxygen (DO), as well as meteorological data (air temperature, wind speed, barometric pressure). Aqueous carbon dioxide concentration was measured at 1 m using a Vaisala® GMT2220 probe modified for water measurements (Johnson *et al.*, 2009). Partial pressure of carbon dioxide (pCO₂) was determined using temperature, depth, and pressure corrections described in Johnson et al. (2009). Specifically, because pressure and temperature respectively increase and decrease sensor output relative to their calibration, measurements were reduced by 0.15% per unit increase hPa relative to calibration (1013 hPa), and increased 0.15%

per unit hPa decrease. An additional correction for depth was added to the barometric pressure correction, because pressure is increased 9.81 hPa per 10 cm depth. Measurements were taken at 1 m, equivalent to a 98.1 hPa increase. Similarly, measurements were increased by 0.3% per degree Celsius increase in water temperature above instrument calibration (25°C).

All water chemistry was performed in the Iowa State Limnology Laboratory. Total nitrogen was determined using the second derivative method described in Crumpton *et al.* (1989). Total phosphorus was determined colorimetrically using the molybdate blue method (APHA, 2012). Samples for Chl a analysis were filtered onto GF/C filters which were frozen then extracted and sonicated in cold acetone under red light. Samples were then analyzed fluorometrically (Arar & Collins, 1997; Jeffrey *et al.*, 1997). Alkalinity was determined by acid titration and reported as mg CaCO₃ L⁻¹ (APHA, 2012). Field measurements of temperature, DO, pH, and conductivity were taken with a YSI multi-parameter probe.

Samples were analyzed by standard isotope ratio mass spectrometry methods (IRMS), and are reported relative to the Vienna Pee Dee Belemnite in ‰ (Equation 1).

$$\delta^{13}\text{C}_{\text{Sample}} = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sample}}/({}^{13}\text{C}/{}^{12}\text{C})_{\text{VPDB}} - 1] \times 1000 \quad \text{Eq. 1}$$

Samples collected for isotopic analysis of dissolved inorganic carbon ($\delta^{13}\text{C}_{\text{DIC}}$) were filtered to 0.2 µm in the field using a syringe filter and cartridge containing a pre-combusted GF/F prefilter (Whatman) and 0.2 µm polycarbonate membrane filter (Millipore). Samples were then injected into helium gas-flushed septa-capped vials with H₃PO₄ to cease biological activity and to sparge CO₂ (Raymond & Bauer, 2001; Beirne *et al.*, 2012). $\delta^{13}\text{C}_{\text{DIC}}$ samples were measured via a Finnigan MAT Delta Plus XL mass spectrometer in continuous flow mode connected to a Gas Bench with a CombiPAL autosampler. Reference standards (NBS-19, NBS-18, and LSVEC) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale.

Average analytical uncertainty (analytical uncertainty and average correction factor) was ± 0.06 ‰.

To determine the isotopic composition of phytoplankton organic carbon ($\delta^{13}\text{C}_{\text{phyto}}$), samples were filtered onto pre-combusted GF/C filters. Zooplankton and detritus were removed manually from filtered samples using a dissecting microscope. Samples were gently fumed in a desiccator for 24 h with 1N HCl to remove inorganic carbon, dried in a low temperature oven, then pulverized using a mortar and pestle and analyzed with standard methods (above IRMS connected to a Costech Elemental Analyzer). For organic isotope samples, three reference standards (caffeine [IAEA-600], cellulose [IAEA-CH-3], and acetanilide [laboratory standard]) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale. The average combined uncertainty for $\delta^{13}\text{C}$ was ± 0.17 ‰ (1 sigma, VPDB). For all isotopic measurements, at least one reference standard was used for every six samples.

Photosynthetic fractionation factors were calculated using published temperature dependent fractionation factors between carbon species following methods described in Trumborn et al. 2009 (Mook, 1986; Trumborn *et al.*, 2009); inorganic carbon fractions and total DIC concentration were calculated using discrete CO_2 , alkalinity, and pH measurements:

$$\delta^{13}\text{C}_{\text{HCO}_3^-} = \frac{\delta^{13}\text{C}_{\text{DIC}} [\text{DIC}] - (\epsilon_a [\text{CO}_2] + \epsilon_b [\text{CO}_3^{2-}])}{(1 + \epsilon_a * 10^{-3}) [\text{CO}_2] + [\text{HCO}_3^-] + (1 + \epsilon_b * 10^{-3}) [\text{CO}_3^{2-}]} \quad \text{Eq. 2}$$

$$\delta^{13}\text{C}_{\text{CO}_2} = \delta^{13}\text{C}_{\text{HCO}_3^-} (1 + \epsilon_a * 10^{-3}) + \epsilon_a \quad \text{Eq. 3}$$

$$\epsilon_p = (\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{phyto}}) / (1 + (\delta^{13}\text{C}_{\text{phyto}} / 1000)) \quad \text{Eq. 4}$$

To test the hypothesized relationships between phytoplankton isotopic composition, photosynthetic fractionation, and ambient pCO_2 (n=196), we used a nonlinear dynamic

regression and ran 199 model iterations (SigmaPlot 12, Systat Software) resulting in 100% model convergence. The same approach was used to test the relationship between photosynthetic fractionation (ϵ_p) and the isotopic composition of the DIC pool. The relationship between phytoplankton biomass as chlorophyll *a* (Chl *a*) and phytoplankton isotopic composition was analyzed using linear regression. Prior to analyses, data were tested for normality using a Shapiro Wilk test.

Results

Phytoplankton $\delta^{13}\text{C}$ signatures in this study ranged from -29.86 ‰ to -13.48 ‰ with an average -25.26 ± 2.8 ‰. The highest values were measured when algal biomass peaked (i.e., during blooms). We found a significant positive linear relationship between phytoplankton $\delta^{13}\text{C}$ and phytoplankton biomass ($\mu\text{g Chl } a \text{ L}^{-1}$, $R^2 = 0.35$, $P < 0.001$, Figure 1), suggesting a shift from diffusive to active uptake of inorganic carbon during blooms. Over the course of this study, bloom conditions, defined as $> 40 \mu\text{g Chl } a \text{ L}^{-1}$ (Table 1; Bachmann et al. 2003), were observed in 46% of our observations with varying degrees of intensity.

We found a significant, positive, non-linear relationship between the stable isotopic composition of the DIC pool and photosynthetic fractionation (ϵ_p , $R^2=0.72$, $P<0.001$, Figure 2). Specifically, the lowest ϵ_p was observed when the $\delta^{13}\text{C}_{\text{DIC}}$ values were less than -8 ‰, or atmospheric levels. Below this level, ϵ_p decreased exponentially toward zero.

Table 1. Summary data for lakes included in this study. Total phosphorus (TP), total nitrogen (TN), and chlorophyll a (Chl a) are reported as average values of all sampling events (ice free season, April to November 2012) \pm standard deviation.

Lake	Latitude	Longitude	TP ($\mu\text{g L}^{-1}$)	TN (mg L^{-1})	Chl a ($\mu\text{g L}^{-1}$)
Arrowhead	42.297218	-95.051228	25 ± 8	0.8 ± 0.1	10 ± 6
Badger	42.586161	-94.192562	58 ± 35	9.4 ± 5.7	33 ± 34
Beeds	42.770320	-93.236436	75 ± 48	7.4 ± 4.5	48 ± 40
Big Spirit	43.479377	-95.083424	46 ± 22	1.1 ± 0.3	22 ± 22
Black Hawk	42.296334	-95.029191	225 ± 118	2.4 ± 0.5	78 ± 35
Center	43.412607	-95.136293	104 ± 50	1.8 ± 0.2	41 ± 36
East Osceola	41.032548	-93.742649	195 ± 77	1.9 ± 0.4	80 ± 47
Five Island	43.145274	-94.658204	106 ± 50	2.1 ± 0.3	67 ± 37
George Wyth	42.534834	-92.400362	62 ± 22	1.0 ± 0.2	26 ± 7
Keomah	41.295123	-92.537482	106 ± 105	1.4 ± 0.6	44 ± 52
Orient	41.196669	-94.436084	397 ± 286	2.3 ± 1.2	144 ± 105
Lower Gar	43.352299	-95.120186	95 ± 35	1.6 ± 0.2	50 ± 23
Rock Creek	41.736936	-92.851859	115 ± 44	1.7 ± 0.4	52 ± 49
Silver (Dickinson)	43.439162	-95.336799	161 ± 85	2.1 ± 0.9	35 ± 58
Silver (Palo Alto)	43.030775	-94.883701	339 ± 206	2.5 ± 0.6	117 ± 60
Springbrook	41.775930	-94.466736	38 ± 25	1.8 ± 0.9	17 ± 14

To evaluate the predicted shift in algal carbon assimilation strategies below atmospheric equilibrium, we used a nonlinear dynamic model to analyze the relationships between ambient pCO_2 and $\delta^{13}\text{C}_{\text{phyto}}$ across lakes and sampling events. We found that while no relationship existed between these variables above atmospheric equilibrium, there was a rapid, significant increase in $\delta^{13}\text{C}_{\text{phyto}}$ (Figure 3; $R^2=0.58$, $P<0.001$) and decrease in fractionation (Figure 4, $R^2=0.66$, $P<0.001$) as CO_2 was depleted below atmospheric equilibrium (393 ppm, NOAA Earth System Research Laboratory, <http://www.esrl.noaa.gov/>).

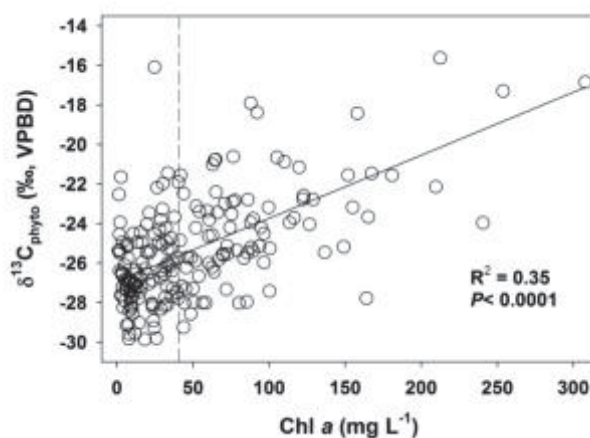


Figure 1. Linear relationship between phytoplankton $\delta^{13}\text{C}$ and chlorophyll *a*, indicating isotopic enrichment increased with phytoplankton biomass. Dashed line indicates phytoplankton bloom conditions, defined here as $>40 \mu\text{g Chl } a \text{ L}^{-1}$ (Bachmann *et al.*, 2003).

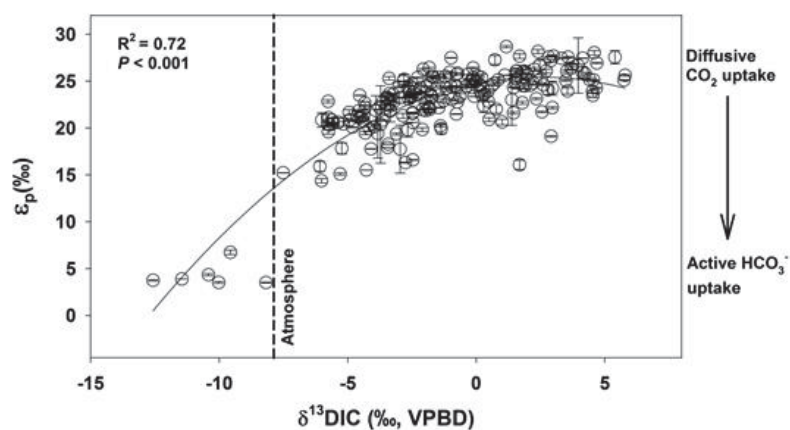


Figure 2. Relationship between the stable isotopic signature of the ambient DIC pool and photosynthetic carbon fractionation. Vertical line indicates an atmospheric DIC source (-7.8 ‰ , VPDB).

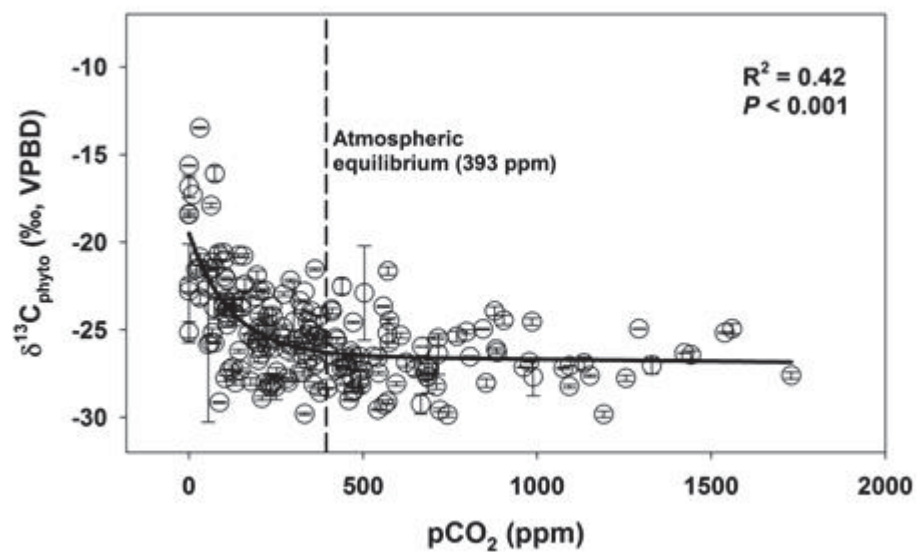


Figure 3. Relationship between the stable isotopic ambient pCO_2 concentration in surface water and the stable carbon isotopic signature of the phytoplankton community. The vertical line indicates atmospheric equilibrium when samples were collected (393 ppm).

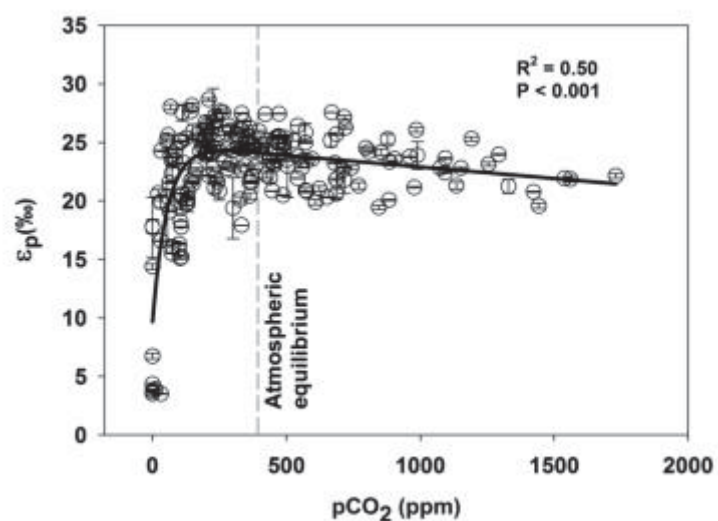


Figure 4. Relationship between photosynthetic fractionation (ϵ_p) and pCO_2 . Vertical line indicates atmospheric CO_2 equilibrium when study was conducted (393 ppm).

Discussion

Our results indicate that alternative carbon assimilation strategies may be an important mechanism sustaining HCBs in anthropogenically eutrophic and hypereutrophic lakes. While previous studies found no predictive relationship between ambient $p\text{CO}_2$ and photosynthetic fractionation (Bade *et al.*, 2006), others have shown long term relationships between $p\text{CO}_2$ and the isotopic composition of phytoplankton (Smyntek *et al.*, 2012). Here we demonstrate that the relationship between $p\text{CO}_2$ and photosynthetic fractionation exists only when $p\text{CO}_2$ drops below atmospheric equilibrium during blooms. We found a similar clear breakpoint below atmospheric equilibrium between $p\text{CO}_2$ and phytoplankton isotopic composition, together suggesting that CCM mechanisms are switched on in phytoplankton communities when ambient water column CO_2 is depleted below atmospheric levels.

The range of values for both $\delta^{13}\text{C}_{\text{phyto}}$ and ϵ_p associated with these trends is consistent with previous laboratory and marine field studies demonstrating shifts from diffusive to active inorganic carbon assimilation via CCM activation (Erez *et al.*, 1998; Cassar, 2004; Trimborn *et al.*, 2009; Boller *et al.*, 2011). Calculated photosynthetic fractionation was lowest during blooms, consistent with phytoplankton CCM utilization. While other freshwater studies have demonstrated similar variability in phytoplankton isotopic composition (Vuorio *et al.*, 2006), ours is the first to demonstrate the co-occurrence of decreased fractionation with CO_2 depletion during blooms in eutrophic and hypereutrophic lakes. This mechanism likely provides a competitive advantage to bloom-forming taxa when high productivity depletes ambient CO_2 .

The $\delta^{13}\text{C}_{\text{DIC}}$ values presented in Bade *et al.* (2006) were more negative than those measured in our study. This difference is attributable to heterotrophic degradation of terrestrial organic matter in northern temperate oligotrophic to mesotrophic lakes (Bade *et al.*, 2007). In our

study, $\delta^{13}\text{C}_{\text{DIC}}$ was relatively enriched in ^{13}C across all lakes and sampling events, with values ranging from -12.5 to + 5.8 ‰, within the range of previously measured values for eutrophic lakes in the same region (de Kluijver *et al.*, 2014). Values in this range can be attributable to mineral dissolution and geochemical fractionation of HCO_3^- at high pH values (Mook 1986; Boutton 1991; Bade *et al.* 2004), and to biogenic methane production via acetate fermentation (Stiller & Magaritz, 1974; Drimmie *et al.*, 1991; Simpkins & Parkin, 1993). In oligotrophic and mesotrophic lakes, these differences correspond to greater average photosynthetic fractionation. In eutrophic and hypereutrophic lakes, however, fractionation decreases with active uptake of mineral bicarbonate (Sharkey & Berry, 1985).

We found a significant positive relationship between photosynthetic fractionation and $\delta^{13}\text{C}_{\text{DIC}}$, which is opposite of what is generally expected in lakes. In other words, fractionation is expected to increase with decreasing $\delta^{13}\text{C}_{\text{DIC}}$ values. Across trophic gradients (e.g., $\delta^{13}\text{C}_{\text{DIC}}$ values between -30 ~ + 5 ‰, Bade *et al.* 2004; de Kluijver *et al.* 2014, this study), these relationships would be driven by decreased $\delta^{13}\text{C}_{\text{DIC}}$ with increasing biomass (i.e., blooms), and decreased fractionation as CCMs are induced (Sharkey & Berry, 1985). In eutrophic and hypereutrophic lakes, however, the range of $\delta^{13}\text{C}_{\text{DIC}}$ values are enriched overall. Our results suggest that CCMs are functioning and fractionation is lowest when the DIC pool is derived from mineral dissolution, and HCO_3^- is the predominant species (~ -15 to 0 ‰, Boutton 1991). Fractionation increased in these lakes as $\delta^{13}\text{C}_{\text{DIC}}$ became more positive, possibly indicating a groundwater –sourced CO_2 generated from organic acid decomposition prior to microbial methanogenesis (Simpkins & Parkin, 1993).

In eutrophic lakes, both phytoplankton isotopic composition and fractionation appear to be strongly related to pCO_2 availability below a critical equilibrium point. In less productive

northern temperate lakes, however, CO₂ is a poor predictor of photosynthetic fractionation (Bade *et al.*, 2006). Our lowest modeled fractionation values reflected active uptake of HCO₃⁻, supported by elevated phytoplankton isotopic values. In contrast, northern temperate lakes had a narrower range of phytoplankton isotopic composition (more negative on average), and much higher ambient CO₂ concentrations, both attributable to heterotrophic degradation of terrestrial carbon. These results indicate inorganic carbon availability drives photosynthetic fractionation in eutrophic lakes, but that other processes (e.g., temperature) likely control it in low-nutrient lakes.

Our results have important implications for how HCBs may be sustained in anthropogenically eutrophic systems. It is well established that high nutrient concentrations result in high phytoplankton biomass (Heisler *et al.*, 2008). It is less clear, however, what mechanisms cause variability in timing and duration of blooms among eutrophic and hypereutrophic lakes. CCMs may provide a competitive advantage to cyanobacteria when high primary productivity depletes ambient CO₂. This mechanism may allow blooms to be sustained for weeks to months at a time with negligible concentrations of CO₂ in the water column (Cotovicz *et al.*, 2015). While nutrient reduction is ultimately critical in the prevention of blooms (Heisler *et al.*, 2008; Rigosi *et al.*, 2014), the mechanism presented here provides insight into causes of bloom duration and intensity at high nutrient concentrations.

Our results show that eutrophic lakes function substantially differently than less impacted surface waters. Temperate lakes are generally considered sources of CO₂ to the atmosphere (Tranvik *et al.*, 2009). We demonstrate that phytoplankton CCM use allows dense phytoplankton to grow at low CO₂ and may facilitate extended periods of high primary production, dissolved CO₂ depletion, and atmospheric CO₂ uptake in surface waters. These processes may increase sediment C burial and the export of autochthonous organic C (Heathcote & Downing, 2011;

Pacheco *et al.*, 2014), and potentially increase methane emissions from anoxic sediments (Hollander & Smith, 2001). Our work demonstrates fundamental differences in inorganic carbon utilization between northern temperate and agricultural, eutrophic lakes. Because the extent of impacted, high nutrient lakes is predicted to increase with the food demands of a growing human population (Foley *et al.*, 2005), understanding mechanisms driving carbon cycling in these systems will be critical in evaluating the impact of HCBs on global carbon cycles.

CHAPTER III

EUTROPHICATION DRIVES EXTREME SEASONAL CO₂ FLUX IN LAKE ECOSYSTEMS

Abstract

Lakes process a disproportionately large fraction of carbon relative to their size and spatial extent, so represent an important component of the global carbon cycle. Alterations to lake ecosystem functioning may alter the balance of greenhouse gas flux in these systems. Without eutrophication, lakes function as net sources of CO₂ to the atmosphere via exogenous inputs and degradation of terrestrial organic carbon. In eutrophic lakes, this process can be reversed due to high primary producer demand for CO₂. Using high frequency sensor measurements, we monitored continuous CO₂ flux during the ice-free season in 15 eutrophic lakes. Here we show that eutrophic lakes can continuously absorb CO₂, day and night, for months at a time. We found net CO₂ influx in 5 lakes (-47 to -1865 mmol m⁻²) and net CO₂ efflux in 10 lakes (328 to 11,755 mmol m⁻²). Across lakes, the highest rates of daily efflux occurred during spring and fall mixing. Regardless of whether CO₂ flux was positive or negative, we found that dissolved inorganic carbon (DIC) was never derived from heterotrophic degradation of terrestrial organic carbon. Instead, stable isotopic analyses revealed that DIC was derived from mineral dissolution, atmospheric uptake, and degradation of autochthonous organic carbon. Optical characterization of dissolved organic matter (DOM) revealed an autochthonous organic matter pool, further supporting our isotopic evidence of autochthony. CO₂ influx was correlated with chlorophyll *a*, autochthonous organic matter, total phosphorus, and dissolved organic carbon. Efflux correlated with total nitrogen and humic DOM. Our results demonstrate that primary producer CO₂ uptake can far exceed biological and geochemical CO₂ generation. Additionally, eutrophic lakes that are net sources of CO₂ to the atmosphere can be large sources,

likely due to the mineralization of endogenous organic matter. These findings suggest that anthropogenic eutrophication has substantially altered biogeochemical processing of carbon in the biosphere.

Introduction

Anthropogenic eutrophication is changing the role of lakes in global carbon cycles. Intensification of industrial agriculture has resulted in massive increases in fertilizer use and the extent of irrigated cropland (Foley *et al.*, 2005). Extensive cultivation alters watershed horizontal permeability, and thus the rate, timing concentration, and quality of inorganic nutrients and dissolved organic matter (DOM) exported to downstream aquatic ecosystems (Foley *et al.*, 2005; Petrone *et al.*, 2011; Williams *et al.*, 2015). Collectively, these processes contribute to degradation of water quality, hypoxia, and harmful cyanobacteria blooms (Heisler *et al.*, 2008; Brooks *et al.*, 2016). In the absence of eutrophication, inputs of terrestrial DOM to lakes fuel heterotrophic respiration in excess of primary production (Duarte & Prairie, 2005; Pace & Prairie, 2005). Coupled with watershed inputs of inorganic carbon, this often results in positive net CO₂ efflux from surface waters (Marcé *et al.*, 2015; Weyhenmeyer *et al.*, 2015; Wilkinson *et al.*, 2016). Because a disproportionate amount of lake carbon research is conducted in northern temperate forested lakes, relatively unimpacted ecosystems (Sobek *et al.*, 2005; Balmer & Downing, 2011), the generalization is frequently made that all lakes function as sources of CO₂ to the atmosphere, that these rates are moderate (i.e., <50 mmol m⁻¹ day⁻¹), and that daytime influx is balanced or exceeded by diel respiratory flux. This may not be true, however, of anthropogenically impacted aquatic ecosystems.

Anthropogenically eutrophied freshwater ecosystems differ from less impacted lakes in watershed cultivation and development (Heathcote & Downing, 2011), nutrient concentrations, primary productivity (Heisler *et al.*, 2008; Pacheco *et al.*, 2014), and dissolved organic matter (DOM) quality (Williams *et al.*, 2015). These differences substantially alter how lakes process, store, and export carbon (Downing *et al.*, 2008; Pacheco *et al.*, 2014; Nöges *et al.*, 2016;). Lakes with agricultural and urban catchments have higher microbial processing rates of organic matter than those with forested watersheds, and a greater contribution of microbial-derived, protein-like compounds (Williams *et al.*, 2010, 2015; Petrone *et al.*, 2011) which tend to persist longer than DOM derived from higher plants (Kellerman *et al.*, 2015). Coupled with elevated nutrient concentrations, this can cause inorganic C uptake by primary producers to exceed that produced via heterotrophic respiration. In the absence of large inputs of humic, terrestrial DOM of higher plant origin, it is unclear if exogenous CO₂ inputs and mineral dissolution can support net CO₂ efflux from surface waters when primary production is high.

Dissolved inorganic carbon (DIC) in lake surface waters can be derived from a diversity of sources including equilibration with the atmosphere, heterotrophic respiration, mineral dissolution, and watershed inputs (Bade *et al.*, 2004). The balance between CO₂ produced via these mechanisms and that fixed by primary production determines the net flux of CO₂ between the lake surface and the atmosphere, though net flux is also determined by physical mixing events and weather patterns (Kling *et al.*, 1992; Del Giorgio *et al.*, 2009). Thus, while high rates of primary production may fix large quantities of inorganic carbon, the combined effects of heterotrophy, mineral dissolution, atmospheric uptake, and physical mixing may prevent eutrophic and hypereutrophic lakes from acting as net CO₂ sinks. Alternately, if inorganic carbon contributions from watershed sources and heterotrophy are small relative to primary

production, productive lakes would be expected to maintain continuous negative flux (CO₂ flux into the lake).

The purpose of this study was to investigate the variability in magnitude and duration of CO₂ concentration in eutrophic and hypereutrophic lake ecosystems, and to assess the relative influence of biological and physical parameters on CO₂ flux direction and rate. We determined the source of inorganic carbon pools and quality of dissolved organic matter across 15 eutrophic lakes using stable isotopic and optical methods. Using high frequency pH and temperature measurements, we calculated continuous CO₂ flux over one ice free season in these systems, and partitioned variability in flux attributable to endogenous (i.e., primary production and autochthonous organic matter) or exogenous (watershed inputs) sources. We hypothesized that net CO₂ influx would be correlated with variables associated with endogenous biological mechanisms and that efflux would be driven by mineral dissolution and physical processes rather than the degradation of terrestrial organic matter.

Materials and methods

Site selection and sampling design

Fifteen eutrophic lakes were chosen along an orthogonal gradient of watershed permeability and interannual variability in Cyanobacteria dominance (Table 1). These sites were selected based on long-term survey data from 132 lakes monitored by the Iowa State Limnology Laboratory between 2000 and 2010 (Ambient Lake Monitoring Program: <http://www.iowadnr.gov/Environmental-Protection/Water-Quality/Water-Monitoring>). Lakes were sampled for standard biological, chemical, and physical parameters during the ice-free season of 2012 once at ice out, twice per week between May and June, once per week in July and

August, and once per month until ice-up. Samples for dissolved organic matter (DOM) characterization and stable isotope analysis of dissolved inorganic carbon ($\delta^{13}\text{C}_{\text{DIC}}$) were collected once in April, at every second sampling event between May and July August, and at every sampling event between September and November.

Water quality measurement and analysis

Lakes were sampled at the historic deep point (Table 1). Integrated upper mixed zone water column samples were collected 0.5 to 2 m depth, depending on lake depth, and above the thermocline when present, using a vertical column sampler. Samples were stored in coolers on ice until delivery to the laboratory within 24 hours of collection, then kept at 4°C and processed to a stable state or analyzed fully within 36 hours of collection. Chlorophyll *a* (Chl *a*), total phosphorus (TP), dissolved organic carbon (DOC), and alkalinity (as $\text{mg L}^{-1} \text{CaCO}_3$) were analyzed using standard EPA approved methods. Total nitrogen (TN) was analyzed using the second derivative method (Crumpton *et al.*, 1989) after autoclave digestion with sodium hydroxide and persulfate. Vertical profiles of dissolved oxygen (DO), specific conductivity, temperature, and pH were measured with a YSI multi-parameter sonde.

High frequency pH and temperature sensors were deployed at the deep point of each lake (TempHion pH/ISE/redox sensor probes; accuracy: ± 0.2 °C; 0.2 pH units; 0.1% mV). Measurements of pH and temperature were recorded every 15 minutes during the ice free season (early April through late November 2012) in order to calculate continuous CO_2 flux. For model calibration, discrete measurements of CO_2 were made at each sampling event using a Vaisala GMT220 atmospheric probe modified for aquatic measurements (Johnson *et al.*, 2009). Continuous sensors were calibrated monthly and cleaned to remove biofouling at each sampling event.

High frequency data correction and CO₂ calculation

Continuous pH data were corrected for drift and comparability to discrete measurements by calculating the difference between discrete measured and sensor pH values at each sampling event. These differences were linearly interpolated between sampling events at an hourly interval using the `approx` function in the R base package (R Core Team, 2015). Hourly sensor pH measurements were then adjusted using the interpolated pH difference to increase comparability between sensor and discrete pH readings. The data were then manually inspected for sensor error within each calibration date range. If the adjusted pH were >1 pH unit outside of the discrete range, the data were trimmed using percentiles based on box plot and data summary statistics. These sampling events were fully removed from the dataset. All data manipulation was performed using R base package (R Core Team, 2015).

Continuous aqueous $p\text{CO}_2$ was calculated based on carbonate equilibria using corrected hourly pH and temperature data, and linearly interpolated discrete measurements of alkalinity and conductivity (Stumm & Morgan, 1996). Error surrounding $p\text{CO}_2$ estimates was determined as the average deviation between calculated $p\text{CO}_2$ and measured $p\text{CO}_2$ for each lake. Hourly flux was calculated as described in Balmer & Downing (2011) and Wilkinson *et al.* (2016) using the equation

$$F_{\text{CO}_2(t)} = (\text{CO}_{2(t)} - \text{CO}_{2(eq)}) * k_H * k_{\text{CO}_2(t)} \quad (1)$$

where $\text{CO}_{2(t)}$ is the concentration of CO₂ in surface water at time t , CO_{2eq} is the average atmospheric equilibrium concentration at time of sampling (393 ppm, NOAA Earth System

Research Laboratory, <http://www.esrl.noaa.gov/>), k_H is the Henry's Law constant for CO₂ at time t , and $k_{CO_2(t)}$ is the piston velocity, calculated as k_{600} (Cole *et al.*, 1994).

Stable isotope analysis

To determine the source of the inorganic carbon pool, $\delta^{13}C_{DIC}$ samples were filtered in the field to 0.2 μm and injected into helium gas-flushed septa-capped vials pre-charged with H₃PO₄ to cease biological activity and to sparge CO₂ (Raymond & Bauer, 2001; Beirne *et al.*, 2012). Samples were measured via a Finnigan MAT Delta Plus XL mass spectrometer in continuous flow mode connected to a Gas Bench with a CombiPAL autosampler. Reference standards (NBS-19, NBS-18, and LSVEC) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale. Average analytical uncertainty (analytical uncertainty and average correction factor) was ± 0.06 ‰. Samples were analyzed by standard isotope ratio mass spectrometry methods (IRMS), and reported relative to the Vienna Pee Dee Belemnite in ‰ (Equation 1).

$$\delta^{13}C_{Sample} = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{VPDB} - 1] \times 1000 \quad \text{Eq. 1}$$

DOM characterization

To assess the source and quality of DOM, water samples were syringe filtered in the field using 0.2 μm pore size polycarbonate membrane filters (Millipore), collected in acid-washed and combusted amber glass bottles, and stored on ice until returning to the lab, then stored at 4°C until analysis. Samples were optically characterized by generating absorbance spectra and excitation-emission matrices (EEMs, Horiba Aqualog UV-Vis benchtop fluorometer/spectrophotometer). Absorbance scans (240 to 600 nm, 3 nm interval) and fluorescence EEMs (excitation: 240 to 600 nm, 3 nm interval; emission 213.7 to 620.5 nm, 3.28

nm interval) were run simultaneously at a fixed 5 nm bandpass. A Milli-Q water blank was run daily. Sample EEMs were corrected for inner filter effects and instrument bias and then blank subtracted (Cory *et al.*, 2010; Murphy *et al.*, 2010; Williams *et al.*, 2010). EEMs were standardized to Raman Units using the area under the Raman peak from the daily Milli-Q blank scan.

Optical indices were calculated to evaluate DOM source (fluorescence index, FI), level of degradation ($\beta:\alpha$ ratio), and humification (humification index, HIX). FI, modified from McKnight *et al.* (2001) was calculated as the ratio of emission at 470 nm to emission at 520 nm at an excitation of 370 nm, and is an indicator of DOM source material (terrestrial or microbial). The $\beta:\alpha$ ratio, an indicator of DOM degradation, was calculated as described in Parlanti *et al.* (2000) and Wilson & Xenopoulos (2008). HIX was calculated as the ratio of peak area under emissions 434-480 nm and 300-346 nm at 255 nm excitation (Zsolnay *et al.*, 1999), with corrections described in Ohno (2002).

Statistical analysis

We assessed covariation between CO₂ flux and environmental predictor variables using partial least squares (PLS) linear regression analysis. The multivariate dataset included indicators of DOM quality (FI, $\beta:\alpha$, HIX) and DIC source ($\delta^{13}\text{C}_{\text{DIC}}$), physical and meteorological data expected to be correlated with CO₂ flux (wind speed, rainfall from <https://mesonet.agron.iastate.edu/>, thermocline depth, lake depth, average epilimnion temperature), and known correlates of autochthonous production (Chl *a*, TN, TP, DOC, DO concentration). CO₂ flux, Chl *a*, $\delta^{13}\text{C}_{\text{DIC}}$, water temperature, wind speed, rainfall, DO, TN, TP, DOC, and thermocline depth were log₁₀ transformed, and DOM quality indices were square root transformed to better approximate a normal distribution and homogenize variance. Variables

having negative estimates ($\delta^{13}\text{C}_{\text{DIC}}$, flux) were added to the absolute value of their minimum measurement prior to log transformation ($\log_{10}(x + |\text{min}|)$). Transformed data were scaled prior to PLS regression analysis.

Results

Water quality and meteorology

Water quality data are summarized in Table 1 and Supplementary Table 1. Across the study period, TN ranged from 0.2 mg L⁻¹ in Lake Orient in July, to 17.1 mg L⁻¹ in Badger Lake in May. With the exception of Arrowhead, Badger, and Springbrook Lakes (7 to 33 µg TP L⁻¹, 7 to 88 µg L⁻¹, and 10 to 89 µg L⁻¹, respectively), all sampling sites had operationally eutrophic or hypereutrophic TP concentrations across sampling events, ranging from 27 µg L⁻¹ in Beeds Lake to 885 µg L⁻¹ in Lake Orient. The highest DOC concentrations were measured in October in Blackhawk Lake (14.6 mg L⁻¹), and the lowest in April in East Lake Osceola (2.7 mg L⁻¹). 2012 was a severe drought year in the Midwestern U.S., so average rainfall across sites was minimal (4.82 ± 2.15 mm). Average lake depth was 4.0 ± 1.55 m, and thermocline depth was 0.85 ± 1.26 m (Supplementary Table S1).

Table 1. Summary data for lakes included in this study. Total phosphorus (TP), total nitrogen (TN), chlorophyll a (Chl a), and DOC are reported as average values of all sampling events \pm standard deviation.

Lake	Latitude	Longitude	TP (µg L ⁻¹)	TN (mg L ⁻¹)	Chl a (µg L ⁻¹)	DOC (mg L ⁻¹)
Arrowhead	42.297218	-95.051228	26 \pm 9	0.9 \pm 0.2	11 \pm 6	5.9 \pm 0.50
Badger	42.586161	-94.192562	58 \pm 35	9.4 \pm 5.8	34 \pm 35	4.4 \pm 1.8
Beeds	42.770320	-93.236436	76 \pm 49	7.5 \pm 4.6	48 \pm 40	4.0 \pm 0.8
Black Hawk	42.296334	-95.029191	226 \pm 119	2.4 \pm 0.6	78 \pm 35	9.0 \pm 1.3
Center	43.412607	-95.136293	104 \pm 50	1.9 \pm 0.3	42 \pm 36	10.5 \pm 0.6

Table 1. continued

East Osceola	41.032548	-93.742649	196 ± 78	1.9 ± 0.5	80 ± 48	8.8 ± 1.2
Five Island	43.145274	-94.658204	106 ± 51	2.1 ± 0.4	67 ± 38	7.7 ± 1.8
George Wyth	42.534834	-92.400362	62 ± 22	1.0 ± 0.2	26 ± 7	4.4 ± 0.3
Keomah	41.295123	-92.537482	107 ± 105	1.4 ± 0.7	45 ± 52	6.3 ± 1.2
Orient	41.196669	-94.436084	398 ± 286	2.3 ± 1.3	144 ± 105	7.4 ± 1.3
Lower Gar	43.352299	-95.120186	96 ± 35	1.7 ± 0.3	51 ± 23	7.9 ± 1.2
Rock Creek	41.736936	-92.851859	115 ± 45	1.7 ± 0.5	53 ± 50	4.9 ± 1.2
Silver (Dickinson)	43.439162	-95.336799	161 ± 85	2.2 ± 0.9	35 ± 59	6.9 ± 0.9
Silver (Palo Alto)	43.030775	-94.883701	340 ± 206	2.6 ± 0.6	118 ± 60	8.2 ± 2.1
Springbrook	41.775930	-94.466736	38 ± 26	1.8 ± 0.9	18 ± 14	3.5 ± 0.4

CO₂ flux

The magnitude of net CO₂ flux for the ice-free season was negative for 5 lakes and positive for 10 lakes (Table 2). The largest net efflux for the ice free season was observed in Badger Lake (11,755 mmol m⁻²), and the largest net influx in Lake Orient (-1865 mmol m⁻²). Average daily flux across sites and sampling events ranged from -45.4 to 757.3 mmol m⁻² d⁻¹ (Figure 1). Across lakes, the greatest efflux was observed during spring or fall mixing (Figure 2; Figure S1), while minimum values were most often observed in mid-summer during periods of stable stratification. The longest period of continuous influx (negative flux, day and night) was 73 days in Lake Orient, while the longest period of net efflux (positive flux, day and night) was 96 days in Lake Arrowhead out of 193 days of continuous sampling (Figure 1; Figure S1).

Table 2. Comparative summary of net annual flux rates for lakes in this study and others.

Lake	Trophic status	Flux (mol m ⁻² yr ⁻¹)	Reference
Orient	Eutrophic	-1.87	This study
Lizard Lake	Eutrophic	-1.50	Pacheco et al.
East Osceola	Eutrophic	-1.10	This study
Green Valley Lake	Eutrophic	-1.00	Pacheco et al.
Sterling Price	Eutrophic	-0.56	Jones et al. 2016
Lake Darling	Eutrophic	-0.50	Pacheco et al.
Prairie Rose Lake	Eutrophic	-0.50	Pacheco et al.
Center	Eutrophic	-0.14	This study
Rock Creek	Eutrophic	-0.14	This study
Silver Palo Alto	Eutrophic	-0.05	This study
Agricultural Lakes	Eutrophic	-0.01	Balmer & Downing 2011
Paho	Eutrophic	0.13	Jones et al. 2016
Peter	Eutrophic	0.20	Wilkinson et al. 2016
Lower Gar	Eutrophic	0.33	This study
Bilby Ranch	Eutrophic	0.37	Jones et al. 2016
Tuesday	Eutrophic	0.40	Wilkinson et al. 2016
Marie	Mesotrophic	0.58	Jones et al. 2016
Marceline 1	Eutrophic	0.62	Jones et al. 2016
Nehai Tonkayea	Mesotrophic	0.67	Jones et al. 2016
Green City	Eutrophic	0.75	Jones et al. 2016
Nodaway	Eutrophic	0.82	Jones et al. 2016
Blackhawk Lake	Eutrophic	0.83	Pacheco et al.
Five Island	Eutrophic	0.87	This study
Paul	Mesotrophic	0.94	Wilkinson et al. 2016
Springbrook	Eutrophic	0.96	This study
Hazel Creek	Mesotrophic	1.08	Jones et al. 2016
Brookfield	Mesotrophic	1.33	Jones et al. 2016
Marceline 2	Eutrophic	1.33	Jones et al. 2016
George Wyth	Eutrophic	1.34	This study
New Bethany	Mesotrophic	1.42	Jones et al. 2016
Silver Dickinson	Eutrophic	1.48	This study
Mozingo	Eutrophic	2.31	Jones et al. 2016
Forest	Mesotrophic	2.58	Jones et al. 2016
Harrison Co.	Eutrophic	2.68	Jones et al. 2016
Beeds	Eutrophic	2.83	This study
Lake Frisksjon	Boreal Humic Lake	4.00	Sobek et al. 2006
Arrowhead	Mesotrophic	4.04	This study
Shingobee Lake	Oligotrophic	4.08	Stets et al. 2009
Keomah	Eutrophic	4.31	This study
Blackhawk	Eutrophic	5.48	This study
Badger	Mesotrophic/eutrophic	11.76	This study
Saline Lakes	Oligotrophic, mesotrophic, eutrophic	33.92	Duarte et al. 2008

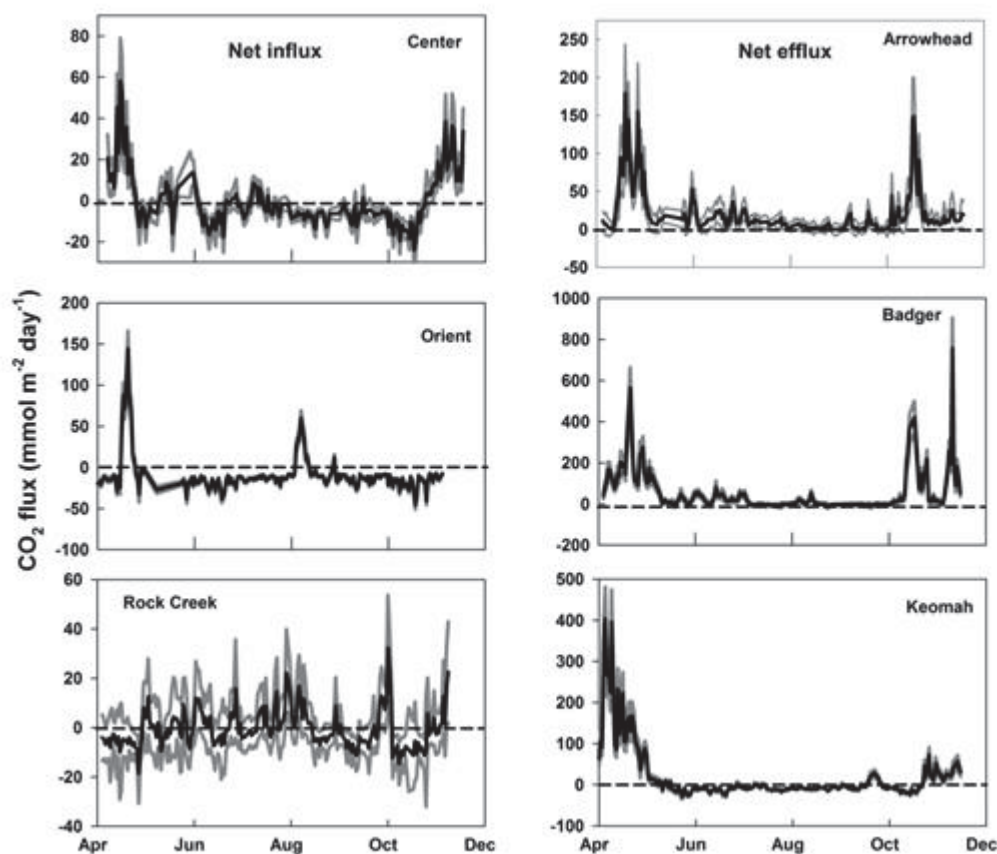
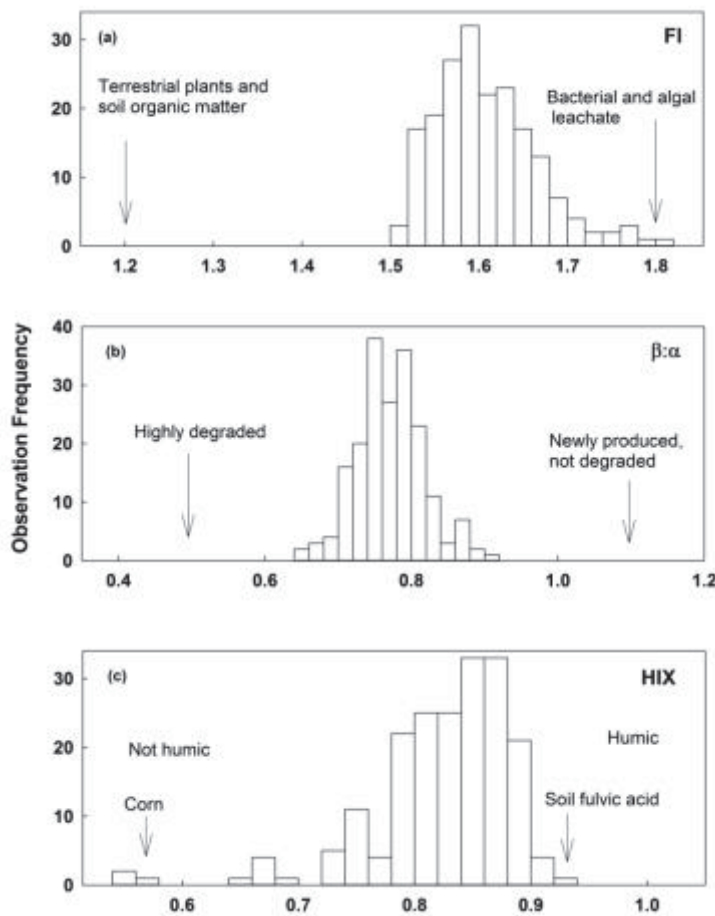


Figure 1. Time series of average daily CO_2 flux ($\text{mmol C m}^{-2} \text{d}^{-1}$) calculated for 6 lakes in this study. Figures in the left column are lakes with net influx during the study period (Center, Orient, and Rock Creek); the right column are lakes with net efflux (Arrowhead, Badger, and Keomah). Black lines are modeled flux; grey lines are 95% confidence intervals. Time series for remaining 9 lakes can be found in Appendix 1.

Organic and inorganic carbon sources

Across seasons and sites, the DOM pool was dominated by autochthonous, degraded organic matter, not of higher plant origin (Figure 2; $\beta:\alpha$: 0.77 ± 0.05 ; FI: 1.6 ± 0.06 ; HIX_{Ohno} : 0.82 ± 0.06). $\beta:\alpha$, an indicator of the level of degradation of the DOM pool, ranged from 0.65 (degraded) in Springbrook Lake in April to 0.91 (newly produced) in Arrowhead Lake in July. FI values ranged from 1.5 to 1.8, and did not show substantial variation across sites or seasons. HIX, indicative of DOM humic content, was between 0.54 (not humic) and 0.92 (humic), with



the lowest values in Blackhawk Lake in August, and highest in Five Island Lake in July. Mean $\delta^{13}\text{C}$ signatures of the ambient DIC pool were -1.16 ± 3.40 ‰, with a range of -12.57 ‰ to 5.78 ‰ (Figure 3). The highest $\delta^{13}\text{C}_{\text{DIC}}$ values were measured in Center Lake in September, and the lowest in Lake Orient in July.

Figure 2. Distribution of DOM quality indices measured in this

study. (a) Fluorescence index (FI). Indicator of DOM source material. Values approaching 1.8 indicate microbial and algal leachate; lower values approaching 1.2 indicate terrestrial organic matter of higher plant origin or soil organic matter. (b) $\beta:\alpha$ ratio. Index of DOM degradation.

Low values indicate highly degraded, processed DOM; high values indicate newly produced DOM. (c) Humification index (HIX). Values approaching 1 indicate humified organic material; lower values are not humic.

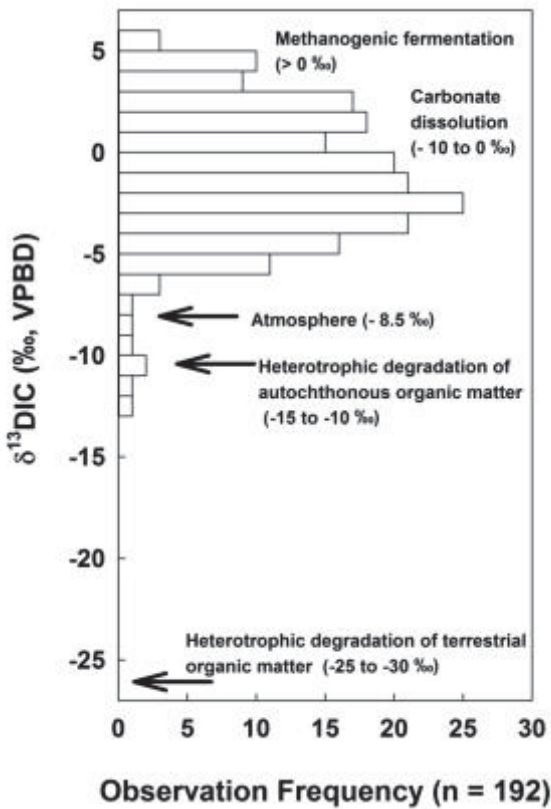


Figure 3. Distribution of isotopic composition of dissolved organic carbon ($\delta^{13}\text{DIC}$) across lakes and sampling events. Values between -25 to -30 ‰ indicate heterotrophic degradation of terrestrial organic matter. -15 to -10 ‰ reflect degradation of bloom organic matter when primary producers are taking up mineral bicarbonate (~ -10 ‰) rather than CO_2 . -8.5 ‰ indicates atmospheric CO_2 . Values between -10 and 0 ‰ and higher reflect mineral dissolution and methanogenic fermentation.

PLS regression

PLS regression analysis revealed relationships with CO_2 flux along gradients of autochthonous production and physical mixing (Figures 4 and 5). As a predictor of CO_2 flux, PLS Component 1 loadings described positive correlations among variables associated with primary productivity (TP, Chl a , DOC, and $\beta:\alpha$), and negative relationships between these and

thermocline depth, HIX, and TN. PLS Component 2 loadings described positive relationships between $\delta^{13}\text{C}_{\text{DIC}}$ and $\beta:\alpha$, which were negatively related to DO, wind speed, and thermocline depth. PLS Component 3 was driven by $\delta^{13}\text{C}_{\text{DIC}}$ which was positively related to wind speed and HIX, and negatively related to thermocline depth and epilimnetic temperature. PLS Component 4 described a positive relationship between FI (DOM source material) and water temperature, which were negatively related to TN. PLS Component 1 explained the most variation in CO_2 flux (Figure 5, $r = 0.55$, $P < 0.001$), describing variability in flux along a gradient of autochthonous production.

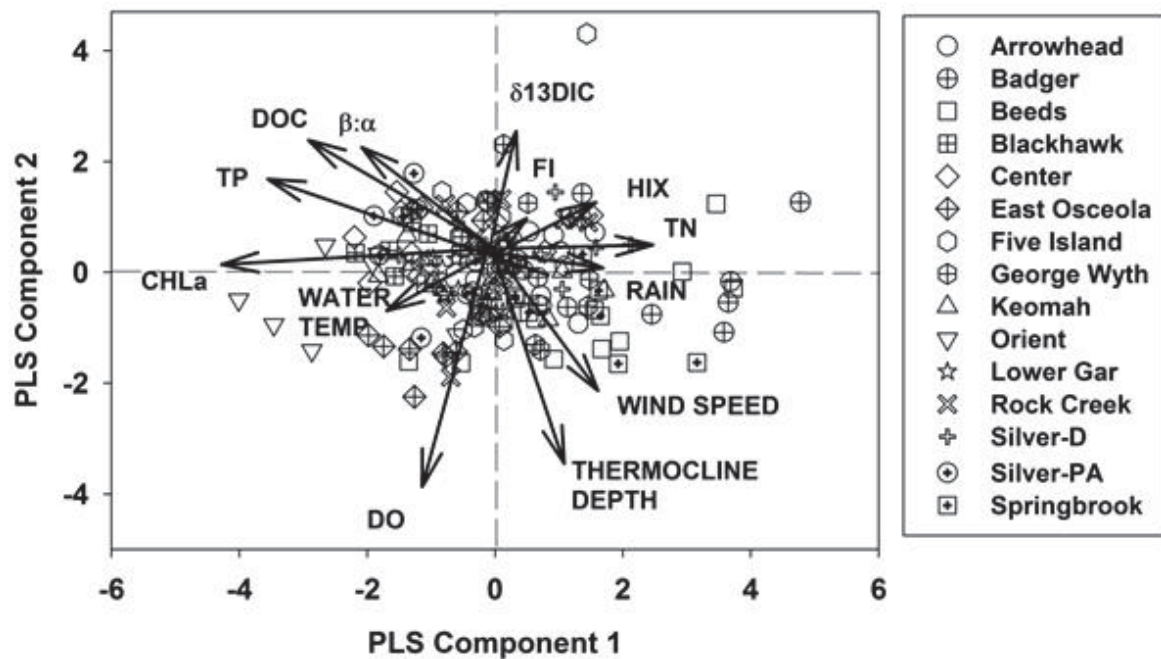


Figure 4. Partial least squares (PLS) regression loadings for PLS Component 1 and PLS Component 2 of discrete measurements illustrating positive correlations among indicators of autochthony (TP, Chl *a*, DOC, and $\beta:\alpha$), which are negatively correlated with TN and DOM humic content (HIX). Shapes reflect individual lakes.

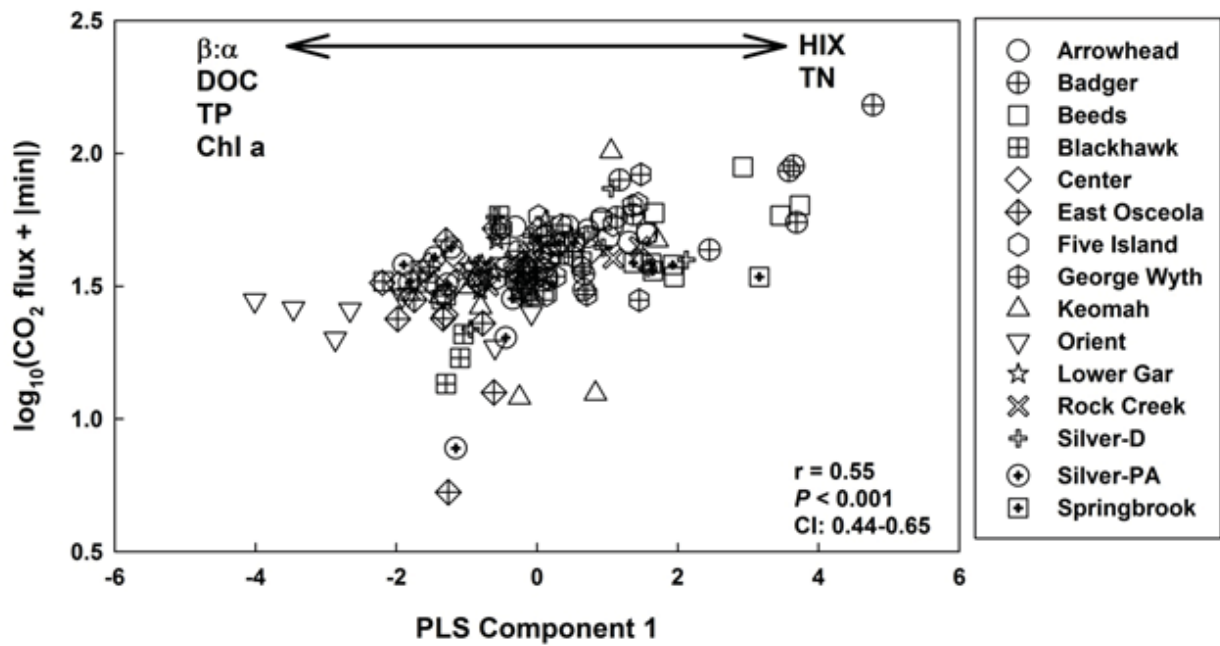


Figure 5. Correlation between CO_2 flux and PLS Component 1, indicating that influx is correlated with indicators of autochthony, and efflux is correlated with TN and HIX. In order to deal with negative values and log transform CO_2 flux to meet assumptions of normality and equal variance prior to analysis, the absolute value of the minimum measurement was added to each data point, then \log_{10} transformed ($\log_{10}(\text{CO}_2 \text{ flux} + |\text{min}|)$).

Discussion

Our results demonstrate that anthropogenically eutrophic and hypereutrophic lakes can maintain negative flux, day and night, for days to months at a time. In these lakes, atmospheric influx was correlated with indicators of autochthonous primary production: total phosphorus concentration, chlorophyll *a*, dissolved organic carbon concentration, and newly produced

dissolved organic matter ($\beta:\alpha$). This suggests that eutrophication processes can fundamentally alter gas flux at the air-water interface.

Across sites and seasons, we found that the organic matter pool was primarily autochthonous. Stable isotope analysis indicated that DIC was derived from the atmosphere and mineral dissolution, never from heterotrophic degradation of terrestrial organic matter. Together, these results show a very different model of lake function than that commonly reported (Cole *et al.*, 1994; Sobek *et al.*, 2005; Raymond *et al.*, 2013; Wilkinson *et al.*, 2016). These findings demonstrate that human activity and eutrophication have not only degraded water quality and altered organic matter composition in lakes (Foley *et al.*, 2005; Li *et al.*, 2008; Williams *et al.*, 2015), but may have much farther reaching effects on CO₂ flux and the role of lakes in global carbon cycles. With increased land use alteration for agriculture and urban centers, more freshwater ecosystems will be subject to these pressures and shift to eutrophic and hypereutrophic states (Foley *et al.*, 2005; Heisler *et al.*, 2008). Our results indicate these alterations can cause dramatic shifts in CO₂ flux rates, both as sustained influx resulting in net CO₂ sinks in some aquatic ecosystems and as very large seasonal efflux in others.

In these lakes, flux was negatively correlated with variables associated with eutrophication and primary productivity. Previous work in experimentally eutrophied ecosystems has suggested that the magnitude of the inorganic carbon demand of autochthonous primary producers will be less than the combined contributions of exogenous watershed CO₂ inputs and heterotrophic degradation of terrestrial organic matter (Wilkinson *et al.*, 2016). While this may be accurate in northern temperate lakes having high contributions of terrestrial plant-derived, humic, and aromatic organic carbon (Sobek *et al.*, 2005; Kothawala *et al.*, 2014), it is not the case in lakes with agricultural watersheds and autochthonous carbon pools. This is evidenced by

previous studies (Balmer & Downing, 2011; Pacheco *et al.*, 2014) and our 5 lakes having net CO₂ influx during the open water season. Optical characterization of DOM in these 5 lakes indicated that their organic matter pools were dominated by compounds resembling newly produced bacterial and algal leachate with low humic content (Supplementary Table S1).

While 5 of the lakes in this study exhibited net CO₂ influx, 10 showed the opposite trend and were net emitters of CO₂. Lakes having the highest rates of efflux scaled with TN, HIX, and rainfall. 2012 was a severe drought year in the Midwestern U.S., resulting in high nitrate accumulation in agricultural soils (Al-Kaisi *et al.*, 2013), which has been shown in many studies to increase nitrate export during rain events (Watmough *et al.*, 2004; Mosley, 2015). Badger Lake, which had more than double the net efflux than any other lake in this study (11,755 mmol m⁻²), also had record high nitrate + nitrite levels in 2012, ranging from 0.4 to 16.8 mg NO₂₊₃-N L⁻¹ with a mean value of 9.4 ± 5.8 mg NO₂₊₃-N L⁻¹. One possible explanation for the co-occurrence of elevated nitrate + nitrite concentrations and flux rates is the photodegradation of nitrate and nitrite in surface waters. This process would generate hydroxyl radicals which mineralize organic carbon, increasing CO₂ efflux (Brezonik & Fulkerson-Brekken, 1998; Molot *et al.*, 2003).

With the exception of Badger Lake, rates of net efflux during the 2012 ice free seasons in these lakes (April 1 to mid-November) ranged from 327 to 5474 mmol m⁻². These values are in a comparable range of previous studies in temperate lakes and reservoirs (Kosten *et al.*, 2010; Barros *et al.*, 2011; Pacheco *et al.*, 2014; Jones *et al.*, 2016), but on average 3 to 4 times higher than previous studies in the same lakes and more recent studies in artificially fertilized northern temperate lakes (Blackhawk Lake, Pacheco *et al.*, 2014; Wilkinson *et al.*, 2016). Calculated flux in Blackhawk Lake reported in Pacheco *et al.* (2014) was determined based on monthly

measurements, compared to high frequency measurements in this study. Discrete daytime measurements do not capture nighttime respiratory flux that high frequency sensors do, and may miss large transient fluxes associated with mixing events. The largest periods of efflux in our study occurred during spring and fall mixing (Figure 1). These periods were not measured in Wilkinson *et al.* (2016), which calculated flux for 100 days between late May and late August, and is one possible explanation for the large difference in net efflux between the two studies. Similar seasonal trends were found by Jones *et al.* (2016) across a gradient of oligotrophic to eutrophic lakes, where the majority annual efflux occurred during spring and fall mixing events.

Stable isotopic analysis of DIC indicated that the DIC pool was derived primarily from mineral dissolution and atmospheric sources, but not from heterotrophic degradation of terrestrial organic matter (Figure 3). Published values of $\delta^{13}\text{C}_{\text{DIC}}$ in lake surface waters generally range from -29.6 ‰ to +2.6 ‰, where the lowest values indicate heterotrophic degradation of terrestrial organic matter (Bade *et al.*, 2004). Depending on proximity to industry and urban areas, atmospheric sources range from -7.5 ‰ to -12 ‰, though the global atmosphere is fairly well mixed and has a nominal value of around -8.5 ‰ (Mook, 1986; Boutton, 1991). If bloom-forming phytoplankton are taking up mineral bicarbonate rather than CO_2 , heterotrophic degradation of autochthonous material should result in $\delta^{13}\text{C}_{\text{DIC}}$ values between -15 and -10 ‰. Values associated with carbonate dissolution typically span from -15‰ to 0‰, however such values (and higher) can also be attributable to sediment methanogenic fermentation in shallow systems (Boutton, 1991). Due to high rates of water column primary productivity, hypolimnetic hypoxia and anoxia, and sediment organic carbon accumulation in these systems (Heathcote & Downing, 2011), methanogenic fermentation is a plausible explanation for elevated $\delta^{13}\text{C}_{\text{DIC}}$ values measured in this study.

Optical characterization of DOM indicated that across lakes, the organic matter pool is composed primarily of endogenous material, both fresh and degraded, with moderate humic content (Figure 3). Average FI values, indicating DOM source material, were 1.6 ± 0.06 . Values approaching 1.2 would indicate terrestrial organic matter of higher plant origin, while values approaching 1.8 are reflective of algal and microbial leachate (McKnight *et al.*, 2001). The average value of $\beta:\alpha$ across sites and sampling events was 0.77 ± 0.05 , suggesting mixed contributions of fresh and degraded material in these lakes. Lower values of $\beta:\alpha$ indicate DOM is highly degraded, while higher values indicate the DOM pool is fresh, or recently produced (Parlanti *et al.*, 2000; Wilson & Xenopoulos, 2008). Similarly, HIX values in our study suggested contributions of humic organic matter in the DOM pool. Because FI values indicate the DOM pool is of microbial and algal origin, and $\delta^{13}\text{C}_{\text{DIC}}$ values do not indicate degradation of terrestrial organic matter is occurring in these systems, these HIX values are likely attributable to microbial humics and reflect rapid processing of endogenous material. These patterns are supported by the $\beta:\alpha$ ratio, which suggests a large portion of the DOM pool in these lakes has been processed and degraded.

Our results indicate that anthropogenic eutrophication can substantially alter carbon biogeochemistry and gas flux in lake ecosystems. While previous work with some exceptions has demonstrated that lakes generally act as net sources of CO_2 to the atmosphere (Cole *et al.*, 1994; Sobek *et al.*, 2005; Wilkinson *et al.*, 2016), we show that inorganic carbon uptake by primary producers can far exceed contributions from heterotrophy and mineral dissolution, rendering lakes net atmospheric CO_2 sinks. We show that many eutrophic lakes maintain negative flux (i.e., continuous CO_2 uptake) for months at a time, meaning high rates of primary production in these impacted ecosystems cannot always be balanced or exceeded by respiration and exogenous

DIC inputs (Wilkinson *et al.*, 2016). Our findings further indicate that these lakes are supported by autochthony, and have minimal contributions of terrestrial organic matter, as evidenced by both optical characterization of DOM and stable isotope analyses. Taken together, these findings suggest that anthropogenic eutrophication is fundamentally changing lake biogeochemistry, gas flux, and their roles in global carbon cycles. As global land use changes to accommodate a large and growing human population, it is likely that more freshwater ecosystems will shift to eutrophic and hypereutrophic states. It will, therefore, be critical to integrate eutrophic and hypereutrophic systems into global carbon budgets and evaluate the effects of these changes at global scales.

CHAPTER IV

NITRITE PHOTODEGRADATION AND ORGANIC MATTER QUALITY CONTRIBUTE
TO ELEVATED CO₂ CONCENTRATIONS IN EUTROPHIC LAKES

Abstract

Recent multi-year drought across the Midwestern United States has resulted in order of magnitude increases in inorganic nitrogen in many freshwater ecosystems. Continuous CO₂ flux data from 16 Iowa lakes collected during 2012 indicated that elevated nitrogen concentrations were temporally correlated with CO₂ super-saturation in surface waters. While carbonate equilibria and biological transformations are responsible for most of the variability in aquatic CO₂ concentrations, photolytic processes yielding reactive oxygen species may also be an important mechanism in the mineralization of organic carbon. In freshwater, the photoreactive species responsible for these reactions are NO₂⁻ and NO₃⁻, chromophoric dissolved organic matter (CDOM), and Fe (II) and (III). Based on these observations, we hypothesized that (1) elevated NO₃⁻ in eutrophic lakes undergoes rapid photolysis resulting in hydroxyl radical production (OH*) and (2) CO₂ flux is directly related to rates of OH* production. During the summer of 2013, we sampled 6 eutrophic lakes with comparable levels of productivity across a gradient of NO₃⁻ concentration. Filtered samples and blanks were spiked with a reference compound known to degrade only in the presence of OH*, then exposed to a natural light regime and sampled over 8 days. First order rate constants for chromophoric decay of the reference compound were not significantly related to initial NO₃⁻ concentration as predicted, but instead were driven only by initial NO₂⁻ concentration ($R^2=0.80$; $P<0.0001$). CO₂ was not directly

related to rate constants, but instead was strongly predicted by the source and quality of the dissolved organic matter pool ($R^2=0.93$, $P<0.0001$; $R^2=0.88$; $P<0.0001$, respectively). These surprising results raise additional questions regarding both terrestrial and aquatic biogeochemical processes that could facilitate increases of this normally transient inorganic nitrogen species.

Introduction

Lake ecosystems in the agricultural Midwestern United States are among the most productive in the world. Anthropogenic pressures associated with a heavily cultivated landscape have resulted in consistently high nutrient concentrations, eutrophication, and frequent harmful phytoplankton blooms. These trends are not isolated to this region, but are of increasing global concern. Most studies of aquatic carbon cycling to date depict freshwater lakes as either at equilibrium with atmospheric carbon dioxide, or as net emitters of CO_2 (Cole et al., 1994; Sobek et al., 2005). Contrary to these findings, recent studies demonstrate CO_2 flux in eutrophic lakes can be highly variable, ranging from extended periods of net influx to high rates of net efflux at comparable levels of primary productivity (Balmer and Downing 2010; Pacheco and others 2014; Jones and others 2016; Morales-Williams et al. 2016, Chapter 3). While sustained net influx is driven by high rates of primary productivity, net efflux can be attributable to both biological and physical processes including net heterotrophy (Pace & Prairie, 2005), exogenous inputs of inorganic carbon (Wilkinson et al., 2016), and the presence of reactive oxygen species which can mineralize organic carbon (Schwarzenbach et al., 2003).

Although carbonate equilibria and biological transformations are responsible for much of the variability in ambient CO_2 in freshwater systems (Stumm & Morgan, 1996), chemical photolytic reactions yielding reactive oxygen species can serve as an additional pathway of

organic carbon mineralization. In freshwater lakes, the photoreactive species responsible for these reactions are NO_2^- and NO_3^- , chromophoric dissolved organic matter (DOM), and Fe (II) and (III) (Schwarzenbach et al., 2003). Because dissolved Fe (II) and (III) should be negligible in the pH range of the lakes we studied (Stumm & Lee, 1960), organic carbon mineralization via the production of reactive oxygen species during NO_2^- or NO_3^- photolysis is a reasonable mechanism that could sustain high ambient CO_2 while production also remains high.

Because reactive oxygen species are transient, short-lived and thus difficult to detect, they are commonly indirectly quantified via chemical kinetics and reaction rates. Their production can be estimated by measuring the disappearance of a reference compound that reacts only by the pathway being tested (Schwarzenbach 2003). If NO_3^- photolysis is occurring, the only reactive oxygen species produced will be OH^* . Erioglaucine, a triphenylmethane compound (Blue Dye No. 1) has been shown to experience loss of chromophoric properties only through interaction with OH^* (Molot et al. 2003). In addition, it has a strong absorbance peak at 620 nm, making its disappearance measureable using absorbance spectroscopy.

Monitoring of several lakes in the agricultural Midwest during the ice free season of 2012 revealed correlations between surface water total nitrogen (TN) and CO_2 concentrations, and an interesting anomaly to expected CO_2 saturation regimes occurred in two of sixteen study lakes. In these lakes, at comparable levels of high primary productivity and autochthonous organic carbon, ambient CO_2 remained super-saturated relative to atmospheric concentrations (Morales-Williams et al. 2016, Chapter 3). This raises the question: why, at high levels of primary production, is ambient CO_2 not drawn down as it is in other eutrophic lakes? The major

difference in these systems relative to others in this study appears to be NO_3^- concentrations orders of magnitude higher than measured in other study lakes (10 to 15 mg L^{-1} versus $< 1 \text{ mg L}^{-1}$, respectively).

The purpose of this study was to investigate NO_2^- and NO_3^- photolysis as potential mechanisms of hydroxyl radical production and organic carbon mineralization, which could sustain CO_2 supersaturation in productive lake ecosystems. Increased nitrogen export from industrial-scale agricultural practices has resulted in increased nitrate (NO_3^-) concentration and export from impacted freshwater ecosystems. This occurs via multiple mechanisms, including transformation and mobilization in surface runoff, via rain events following drought, and accumulation over winter under ice. This has far reaching effects, including local drinking water contamination (Hatfield *et al.*, 2009), increased stream nitrous oxide emissions (Turner *et al.*, 2015), and the Gulf of Mexico coastal dead zone at the mouth of the Mississippi River (Rabalais *et al.*, 2002). While much attention has been given these processes, the implications of increased nitrate loads on lake carbon cycling and CO_2 flux is underexplored. We combined field and experimental methods to investigate whether hydroxyl radical production via NO_2^- and NO_3^- photodegradation is a mechanism of organic carbon mineralization, sustaining CO_2 supersaturation in eutrophic lakes.

Methods

Sample collection and processing

Six eutrophic lakes were chosen along a gradient of nitrate (NO_3^-) concentration (Table 1) based on data survey data collected in 2012 (Figure 2). Triplicate integrated epilimnetic surface water samples were collected from each lake once in May 2013. Water column pCO_2

(Vaisala GMT2220 atmospheric CO₂ probe modified for aquatic measurements; Johnson *et al.*, 2009) and depth profiles of dissolved oxygen, temperature, conductivity, and pH were measured at the time of sampling (YSI multi parameter sonde). Water samples were placed immediately in a dark cooler to avoid light exposure. Upon returning to the lab, samples were filtered to 0.2 µm using combusted GF/F filters (Whatman) paired with sterile polycarbonate membrane filters. 30 mL from each filtered sample was reserved for chemical analyses. All sample processing was conducted under red light.

Chemical analyses

Filtered water samples were analyzed for dissolved organic carbon (DOC), NO₃⁻ and NO₂⁻ concentration, and dissolved organic matter (DOM) quality. DOC was measured using standard EPA approved methods (Shimadzu total organic carbon analyzer). NO₂⁻ was determined colorimetrically using the cadmium reduction method (APHA), and NO₃⁻ was determined using the second derivative method (Crompton *et al.*, 1989). Samples for DOM analysis were optically characterized by generating excitation-emission matrices (EEMs) and absorbance spectra as described in Morales-Williams *et al.* (2016, Chapter 3). Optical indices of DOM source, aromaticity, level of degradation, and level of humification were calculated as described in Morales-Williams *et al.* (2016, Chapter 3) and Williams *et al.* (2015) as follows: a modified fluorescence index describing terrestrial or microbial DOM source material (FI, McKnight *et al.*, 2001; Cory *et al.*, 2010); specific UV absorbance at 254 nm indicating DOM aromaticity (SUVA₂₅₄, Weishaar *et al.*, 2003); the β:α index, a correlate of the level of degradation of the DOM pool (Parlanti *et al.*, 2000); and a modified humification index which describes humic content (HIX, Zsolnay *et al.*, 1999; Ohno, 2002).

Experimental design and analysis

Triplicate filtered water samples and 18 DI blanks (500 mL) were placed in 1L whirlpak bags. Each bag was spiked with erioglaucine dye to reach a concentration of 8.5 mg L^{-1} . HOBO light and temperature loggers (64K UA-002-64) were placed in 6 unspiked bags containing only DI water. Sample bags were randomly distributed using a random number table in 6 circulating tanks, with triplicate control blanks in each tank (Figure 1). Water temperature was set to 21°C , representative of in-lake surface water temperatures at the time of sample collection. Tanks were exposed to a natural light regime in a rooftop greenhouse over 8 days.

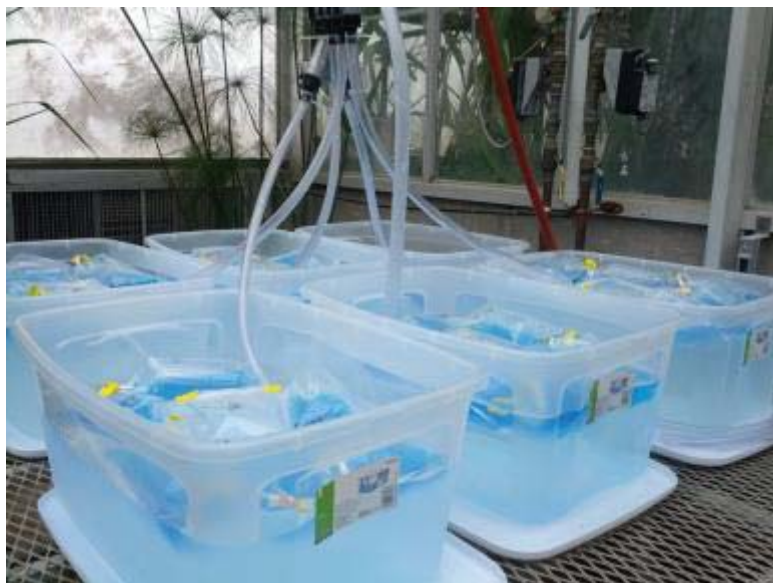


Figure 1. *Experimental circulating tanks containing erioglaucine-spiked samples and blanks. Samples were exposed to a natural light regime in a rooftop greenhouse. Water baths were maintained at ambient temperatures comparable to lake surface water at the time of sample collection.*

The experiment was sampled fitting an exponential decay model 5 times over 8 days: T^0 : initial deployment, T_1 : Day 1, T_2 : Day 2, T_3 : Day 4, and T_4 : Day 8. All sampling and analyses were conducted before dawn under red light. At each sampling event, a 10 mL aliquot of water was removed from sample bags and absorbance was measured 620 nm (Molot *et al.*, 2003).

Data analysis

The exponential decay rate of erioglaucine absorbance at 620 nm (k) was determined from the slope of the linear relationship between the natural log of percent absorbance loss and time over the duration of the experiment for each lake (Figure 3). Because initial concentrations of erioglaucine were the same in all samples and concentrations of all specific scavengers were unknown, k (d^{-1}) was used to compare samples (Molot *et al.*, 2003). In order to determine the primary drivers of hydroxyl radical (HO^*) production during the experiment, relationships between k d^{-1} and predictor variables measured at the time of sample collection (NO_2^- , NO_3^- , DOC, CO_2 , SUVA_{254} , FI, $\beta:\alpha$, and HIX) were tested using univariate regression. In order to assess the contribution of organic matter quality to organic carbon mineralization, we also tested relationships between optical indices and pCO_2 (R Core Team, 2015). Finally, correlations and time series and of total nitrogen (TN) and pCO_2 from 2012 (year preceding this study) were plotted to visualize seasonal temporal patterns between these variables.

Results

In order to visualize covariation between total nitrogen concentrations and ambient CO_2 at study sites in the year preceding this experiment, we plotted time series of TN and pCO_2 concentrations measured on a weekly to bi-weekly basis between ice off and ice on (April to November, Figure 2). Relationships between CO_2 and TN were highly variable between lakes and sampling events. In lakes with the highest average TN concentrations (Badger and Beeds), CO_2 and TN tightly covaried in the spring and summer, but diverged in the fall. We saw similar patterns in the two lakes with the lowest average TN concentrations (Arrowhead and Orient), but with greater variability overall and less coherence in trends in late summer.

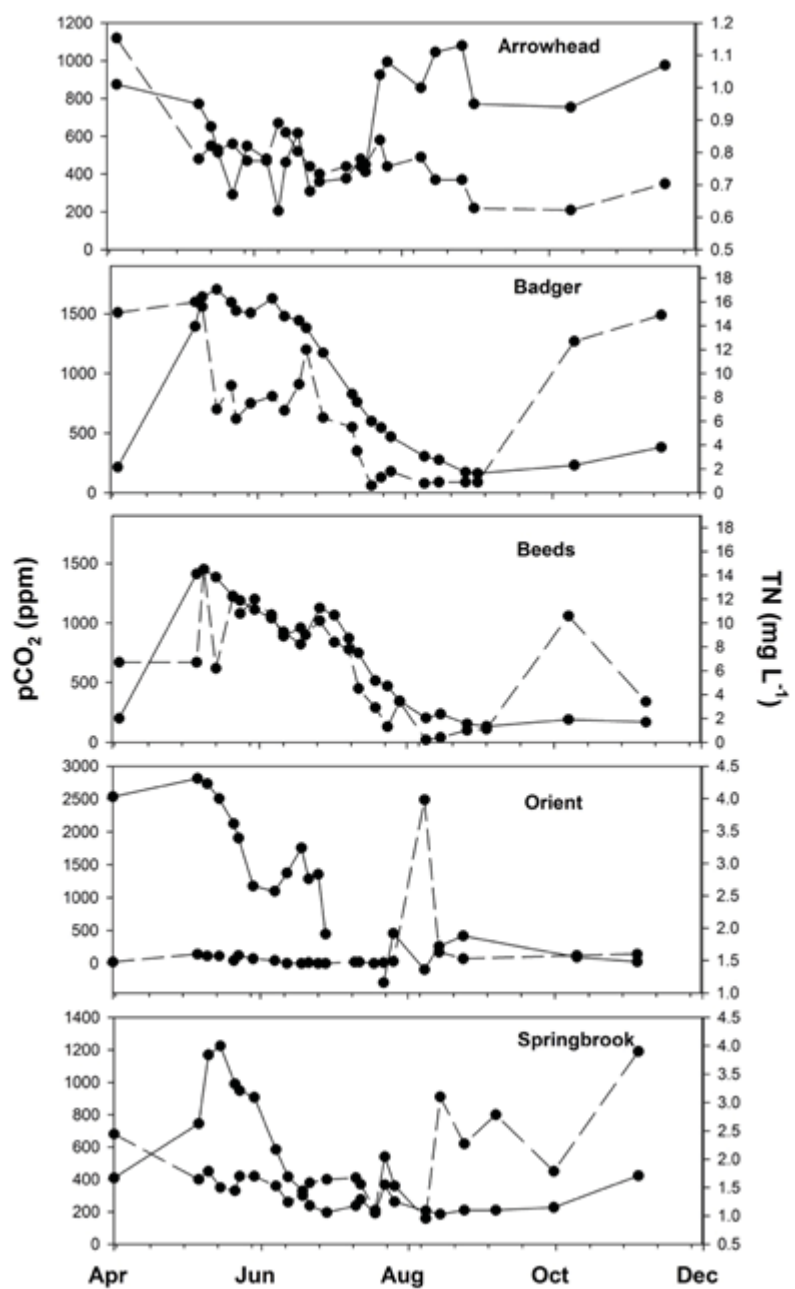


Figure 2. Time series of total nitrogen (TN, mg L^{-1}) and $p\text{CO}_2$ concentration (ppm) during the ice-free season of 2012. Solid lines indicate TN and dashed lines indicate $p\text{CO}_2$.

Exponential decay rates (k) for the loss of erioglaucine absorbance in lake water under a natural light regime ranged from -0.0603 d^{-1} in Orient Lake samples to -0.0013 d^{-1} in Arrowhead (Figure 3).

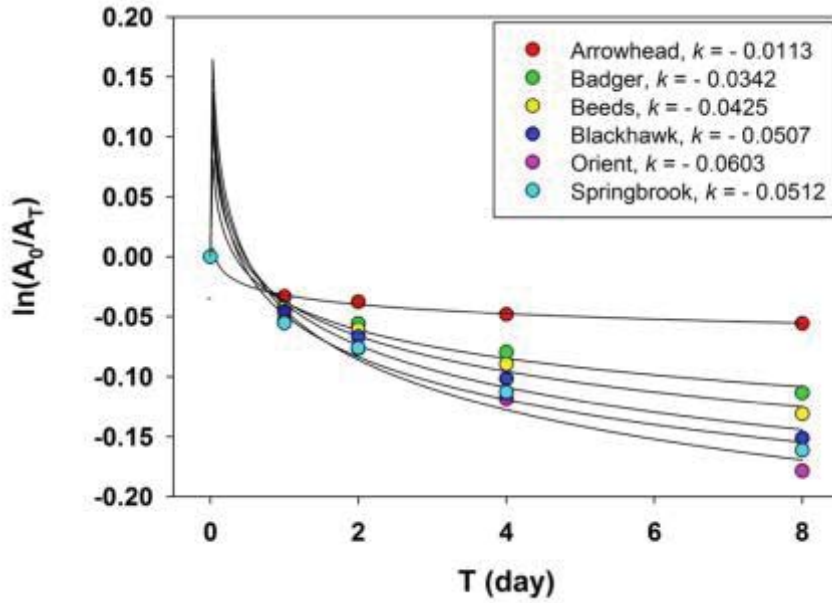


Figure 3. Exponential decay rates (k) of erioglaucine absorbance at 620 nm over the duration of the experiment. A_0 indicates absorbance at 620 nm at time 0; A_T indicates absorbance at the time of

sampling. k is the slope of the slope of the relationship between time of sampling and $\ln(A_0/A_T)$.

Contrary to our predictions, we did not find a direct relationship between loss of absorbance and in-lake CO_2 concentration. Exponential decay rates were only significantly related to initial in-lake NO_2^- (mg L^{-1}) concentration, and no other variables measured in this study, including CO_2 , NO_3^- , DOC, and optical indices (Figure 4).

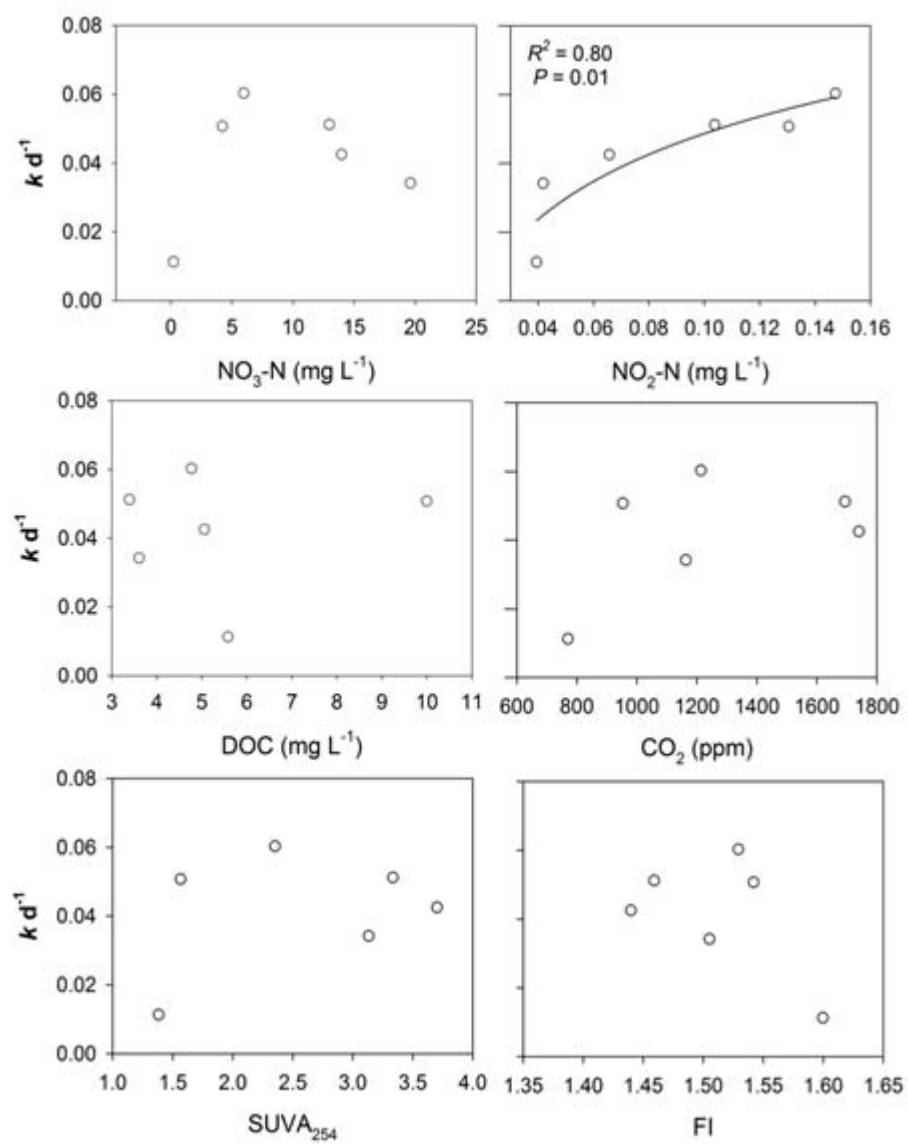


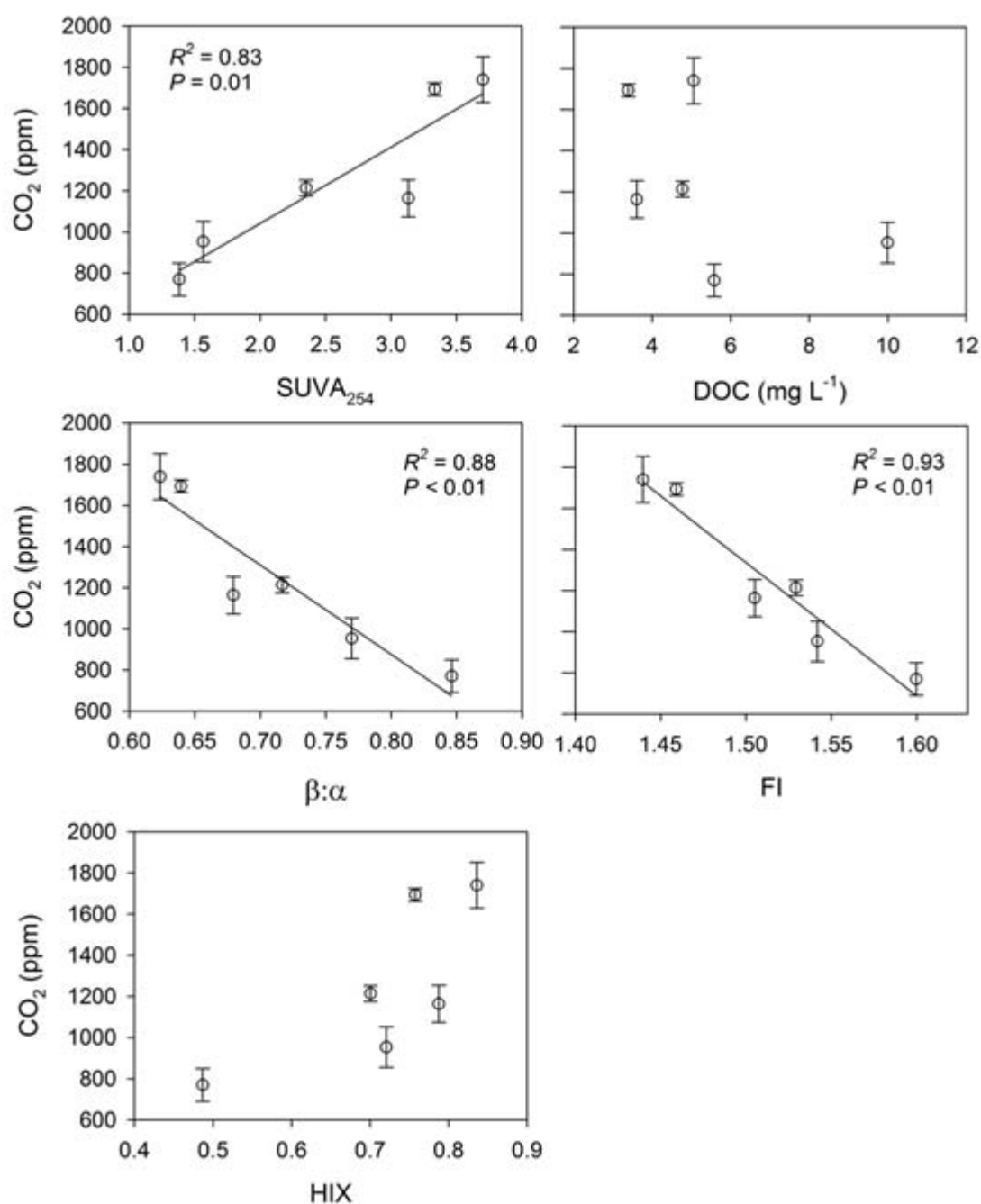
Figure 4. Relationships between exponential decay rates (k d⁻¹), and in-lake variables at the time of sample collection. k was significantly related only to initial nitrite concentration.

Table 1. Sampling sites, locations, and in-lake NO_2^- , NO_3^- , DOC, and CO_2 concentrations at the time of sample collection.

Lake	Latitude	Longitude	NO_2^- (mg L ⁻¹)	NO_3^- (mg L ⁻¹)	DOC (mg L ⁻¹)	CO_2 (ppm)
Arrowhead	42.297218	-95.051228	0.1 ± 0.0	0.2 ± 0.04	5.6 ± 0.3	770 ± 79
Badger	42.586161	-94.192562	0.1 ± 0.0	19.6 ± 0.4	3.6 ± 0.0	1163 ± 90
Beeds	42.770320	-93.236436	0.2 ± 0.1	14.0 ± 0.2	5.1 ± 0.1	1740 ± 111
Blackhawk	42.296334	-95.029191	0.1 ± 0.1	4.2 ± 0.1	10.0 ± 0.2	953 ± 99
Orient	41.196669	-94.436084	0.1 ± 0.2	6.0 ± 0.1	4.8 ± 0.1	1213 ± 38
Springbrook	41.775930	-94.466736	0.1 ± 0.1	13.0 ± 0.1	3.2 ± 0.1	1693 ± 31

While we found no significant relationship between k and ambient pCO_2 , we found that CO_2 was strongly correlated with several indicators of organic matter quality. SUVA_{254} was positively related to pCO_2 ($R^2 = 0.83$, $P < 0.001$), and $\beta:\alpha$ and FI were negatively related ($R^2 = 0.88$, $P < 0.01$; $R^2 = 0.93$, $P < 0.01$, respectively; Figure 5). We found no significant relationships between CO_2 and DOC concentration or HIX.

Figure 5. Regression relationships between ambient CO_2 at the time of sample collection and



optical indices of organic matter quality. SUVA_{254} is specific UV absorbance at 254 nm and is an indicator of DOM aromaticity. Larger values indicate a greater proportion of aromatic moieties in the DOM pool. $\beta:\alpha$ is an indicator of the level of degradation of the DOM pool, where higher

values indicate fresh or newly produced material, and lower values indicate highly degraded DOM. FI is the fluorescence index and is a correlate of terrestrial (lower values) or microbial (higher values) DOM. HIX is a humification index that correlates to the level of humification of the DOM pool.

Discussion

Our experimental results indicate that in these lakes, photolysis of accumulated nitrite, not nitrate, drives hydroxyl radical production, though it is unclear if this process is directly related to carbon mineralization and elevated CO₂ concentrations. We found strong predictive relationships between indices of DOM quality and ambient CO₂, demonstrating that the source and quality of organic matter are important determinants of carbon mineralization in these systems. While these results do not fully align with our predictions, they provide insight into the fate and transformation of nitrogen loads in surface waters of impacted lake ecosystems. Previous work in 2012 demonstrated temporal correlation between TN and lake pCO₂ concentrations, suggesting a relationship may exist between these variables over longer temporal scales that was not captured in our short term study.

We found a significant positive relationship between initial, in-lake NO₂⁻ concentrations and exponential decay rates of eriochlorogenic acid, indicative of HO^{*} production (Figure 3). Under normal conditions, NO₂⁻ is rapidly oxidized to NO₃⁻ via a low energy reaction in aerobic aquatic ecosystems. Both low temperature and high pH, however, can inhibit this process due to tolerance ranges of nitrite oxidizing bacteria, leading to accumulation of NO₂⁻ in surface waters (Wetzel). The combined effect of high nitrogen loads from agricultural runoff and elevated pH resulting from high primary productivity may therefore inhibit efficient NO₂⁻ oxidation to NO₃⁻.

in these systems. In addition to this, NO_2^- compounds can absorb 30 times more light, and have a 4 times greater quantum yield than NO_3^- , which can result in two orders of magnitude greater hydroxyl radical production per mole than NO_3^- (Schwarzenbach *et al.*, 2003). The implications of this are that a very small accumulation of NO_2^- can have a much larger effect on the production of reactive oxygen species and their potential for carbon mineralization than NO_3^- .

While in-lake pCO_2 was not directly related to HO^* production in our study, we found strong relationships between pCO_2 and optical indices of DOM source and quality (Figure 4). SUVA_{254} , and indicator of DOM aromaticity, was a strong positive predictor of pCO_2 , and $\beta:\alpha$ and FI were negatively related. These results indicate pCO_2 concentration in these lakes is tightly coupled with aromatic organic compounds, and negatively related to newly produced, not degraded DOM (indicated by $\beta:\alpha$), and microbial, autochthonous DOM not of terrestrial origin. These results were expected, as highly aromatic, terrestrial DOM is expected to drive heterotrophic respiration and CO_2 supersaturation in lakes (Pace & Prairie, 2005). While we did find a relationship with SUVA_{254} , and indicator of DOM aromaticity (Weishaar *et al.*, 2003), we did not find a relationship with DOC, indicating this pattern was driven primarily by DOM absorbance at 254 nm, not DOC concentration.

Previous investigations of DOM in these systems have demonstrated minimal contributions of terrestrial organic matter, and a dominant autochthonous carbon pool (Morales-Williams *et al.* 2016, Chapter 3). Organic atrazine-based herbicides have been shown to inflate absorbance values at 254 nm (Nick *et al.*, 1992). Because SUVA_{254} is calculated as absorbance at 254 divided by DOC concentration, it is possible that inflated SUVA_{254} values reflect agricultural herbicide loads, not aromatic moieties of terrestrial plant origin. The NO_2^- and NO_3^- photolysis pathways may be complicated by increasing concentrations of organic carbon, particularly highly

aromatic, terrestrially derived compounds. When this is the case, chromophoric DOM acts both as a source and quenching agent of reactive oxygen (Blough, 1998), making understanding DOM source and quality important in interpreting both the inorganic nitrogen and organic carbon photooxidative pathways. It is therefore possible that while nitrogen loads are correlated with DOC mineralization, the dominant mechanism is not photooxidation of inorganic nitrogen, but of organic pollutants likely co-exported to lakes with nitrogen fertilizers.

The purpose of this study was to explore photooxidative mechanisms maintaining CO₂ supersaturation in eutrophic, highly productive lakes. Based on data collected in the year preceding this study demonstrating seasonal covariation between TN and pCO₂ in lake surface waters, we predicted that nitrate from agricultural runoff was being photodegraded yielding hydroxyl radicals responsible for mineralizing organic carbon pools. Contrary to our predictions, we found that NO₂⁻ was the dominant inorganic nitrogen species responsible for HO^{*} production. These results are significant because they indicate accumulation of a usually transient nitrogen species, which may be attributable to inhibition of the microbial nitrification pathway. We did not demonstrate a direct relationship between ambient CO₂ concentration and HO^{*} production. Instead, the best predictors of surface water pCO₂ in these lakes was the source and quality of the chromophoric DOM, demonstrating the importance of understanding the composition of this highly reactive organic matter pool. Because these lakes are autochthonous and have low contributions of terrestrial, lignin-based organic carbon, it is possible that the high SUVA₂₅₄ values measured here are attributable to atrazine-based herbicides. These results demonstrate that eutrophication of inland waters is not limited to phosphorus inputs and algal blooms, but may be fundamentally altering lakes' biogeochemical processing of carbon and nutrients.

CHAPTER V

TEMPORAL DESTABILIZATION OF PHYTOPLANKTON COMMUNITIES VIA
SYNCHRONOUS FLUCTUATION IN THE CYANOBACTERIA

Abstract

A common characteristic of impacted freshwater ecosystems are periodic harmful phytoplankton blooms and cyanobacteria dominance. With global increases in watershed alteration via expanding agriculture and urbanization, eutrophication of inland waters remains an unresolved issue. Primary productivity driven by lake phytoplankton impacts large scale ecosystem processes including gas flux and carbon burial. Changes in biodiversity and function of lake primary producer communities thus can have large scale feedbacks on aquatic ecosystems and elemental cycling. Theory previously tested in terrestrial ecosystems suggests that productivity and temporal stability tend to be positively correlated. In eutrophic lakes however, this relationship can decouple, where the highest rates of primary productivity are sometimes associated with low-diversity, seasonal cyanobacteria blooms. We tested the relationship between synchronous population fluctuation and stability of productivity on two temporal scales: bi-weekly for one year, and annually for 10 years. In our short term study, we found high variability between lakes in cyanobacteria dominance and community composition preceding and during blooms at operationally eutrophic nutrient concentrations. We show that on a long term basis, these cyclic fluctuations in productivity are strongly synchronized with phytoplankton population dynamics, and that stability has a non-linear, negative relationship with genus synchrony. On a short term basis, however, we found that primary producer stability

was greatest at intermediate levels of cyanobacterial synchrony, and lowest at high levels of synchrony. These results suggest that eutrophic lakes are resilient to moderate fluctuation in primary producer populations, and that biodiversity loss resulting from cyanobacteria dominance is an ecological mechanism of instability in lake ecosystems.

Introduction

Theory predicts that environmental stochasticity and large fluctuations in community size due to cyclic or chaotic processes tend to synchronize population dynamics. These patterns are expected to occur primarily as a result of density dependence, and may proceed due to internal or external forcing mechanisms (Loreau & de Mazancourt, 2008). In terrestrial systems, synchronous fluctuations between population and community size have been shown to be negatively related to temporal stability of productivity (Isbell *et al.*, 2009; Hector *et al.*, 2010). These findings are consistent with previous work by Tilman and others (1998) that predicted temporal stability could be maintained under asynchronous fluctuations if ecological function remains constant. Further, greater species diversity and evenness should support temporal stability and resilience to perturbation via a large species pool able to differentially appropriate key ecological functions (Tilman & Downing, 1994).

The theoretical framework predicting synchronous or compensatory dynamics in grasslands is equally relevant in aquatic ecosystems. Mechanisms maintaining stable coexistence of phytoplankton species have been proposed and revisited for decades, and it is generally accepted that environmental variability and spatiotemporal niche partitioning can account for this paradox (Hutchinson, 1961; Descamps-Julien & Gonzalez, 2005; Fox *et al.*, 2010). A few empirical studies have examined synchrony-stability relationships in the phytoplankton, and

have produced variable results. Like in grasslands, diverse phytoplankton communities tend to exhibit compensatory dynamics under moderate stress, such as salinity gradients (Flöder *et al.*, 2010). In controlled laboratory studies, however, functionally similar populations exhibit a greater degree of synchrony than functionally diverse assemblages (Rocha *et al.*, 2011).

Additionally, multiple studies have found that these patterns are strongly dependent on temporal scale due to the rapid generation times and seasonal succession of unicellular organisms. Trends therefore may be diluted or undetectable when analyzed at annual scales (Vasseur *et al.*, 2005; Rocha *et al.*, 2011), making it important to assess community phenology on time scales relevant to the growth and turnover rate of the community being studied.

Bloom-forming Cyanobacteria communities represent a relevant model system to test synchrony-stability theory. Because these blooms are a frequent seasonal occurrence in impacted lakes (Heisler *et al.*, 2008), they would be expected to exhibit high levels of synchrony with effects on the phytoplankton community and ecosystem processes when they are dominant. Additionally, similar to terrestrial invasive species, they can maintain very high levels of productivity and biomass under relatively low levels of diversity, though these patterns may vary when bloom phenology is examined at different time scales, and with the dominant taxa or functional group forming the bloom. In this context, questions remain as to whether seasonal bloom dynamics are a mechanism of temporal stability via maintenance of productivity, or instability via low diversity and high dominance in impacted lakes.

The role of disturbance in community assembly has been widely explored both theoretically and empirically across many types of ecosystems, including tropical rainforests, grasslands, coral reefs, and wetlands (Connell, 1978; Levins, 1979; Odum *et al.*, 1979; Pimm, 1984; Ives, 1995; Yachi & Loreau, 1999; Chesson, 2000). Studies of lake phytoplankton,

however, in general remain firmly rooted in equilibrium theory (Tilman *et al.*, 1982; Sterner, 1989; Naselli-flores *et al.*, 2003), with notable recent exceptions (Anneville *et al.*, 2005; Spatharis *et al.*, 2007; Beaver *et al.*, 2012). This framework predicts phytoplankton will assemble by competitive exclusion in a predictable way along spatial and temporal resource gradients (Descamps-Julien & Gonzalez, 2005), and that these patterns will predictably vary among lakes of different trophic status. This has been demonstrated theoretically and empirically, and has become the textbook explanation of what phytoplankton do. In eutrophic lakes however, variability in community structure at comparably high ambient nutrient concentrations is not fully explained by equilibrium theory, and may instead be driven by small scale fluctuation rather than average, equilibrium conditions (e.g., Yachi and Loreau 1999; Loreau 2000; Chesson and others 2004).

In eutrophic lakes, large fluctuations in primary producer biomass alter biogeochemical carbon cycling and fluxes, corresponding to synchronous fluctuations in carbon dioxide influx and efflux (Morales-Williams *et al.* Ch. 4). Synchronous fluctuation in phytoplankton communities due to bloom events thus have the potential to significantly alter ecosystem function by facilitating extreme flux events comparable to ecosystem scale hyperventilation, with implications for the role of lakes in global carbon cycles. It is unclear, however, if these trends vary with the diversity of bloom communities, and whether monospecific blooms have a greater impact on ecosystem function via synchronous fluctuation than multi-specific ones. The purpose of this study was to first investigate drivers of variability in phytoplankton community phenology and structure in three eutrophic lakes biweekly over one year. Second, we investigated relationships between phytoplankton community synchrony and temporal stability at both annual and monthly time scales. We predicted that at the genus level, both long and short

term cyclic population dynamics of bloom formation are synchronized with phytoplankton community abundance, and synchronous fluctuations in the cyanobacteria act to destabilize the temporal stability of phytoplankton communities.

Methods

To test these hypotheses, we analyzed (1) a long-term taxonomic dataset from twenty mesotrophic to hypereutrophic lakes, collected 3 times per year as part a lake monitoring program (Appendix 2) and (2) a short term dataset comprised of bi-weekly to monthly samples collected in three eutrophic lakes during the ice-free season of 2012. Limnological data and analytical methods for these lakes (Keomah, Orient, and Springbrook) can be found in Chapter 3 and Appendix 1. Long-term samples were collected during the ice free seasons between 2000 and 2011. For this dataset, each sampling event was treated as discrete and independent rather than as a yearly average in an effort to avoid temporal scale dilution of synchronous or asynchronous effects. For consistency, 2008 and 2011 were excluded from analyses due to incomplete or missing data. Additionally, years 2000 and 2001 were excluded as different methods were used to calculate phytoplankton biovolume. All biovolume data included in this study were calculated using methods presented in Hillebrand and others (1999). Phytoplankton taxa were identified at the genus level, and comprise 106 genera across the large dataset.

Phytoplankton samples were identified and enumerated using either an upright or inverted compound microscope scope and settling chamber (long term data) or nanoplankton counting chamber (short term data). Long term samples were counted and identified at 200x magnification, short term were counted at 400x, and identified at either 400 or 1000x. Samples

were counted to 300 natural units, defined as a colony, filament, or single cell if not colonial. Biovolume measurements were taken of at least 30 representative cells within 10 colonies per sample, and at least 10 colonies, filaments or single cells of each genera if present.

The Shannon index was used to estimate α -diversity in short term samples in order to account for both richness and evenness. To analyze variability in synchrony and compensatory dynamics across sampling events, we calculated an index of community wide synchrony as presented in Loreau and de Mazancourt (2008; R Core Team 2015) using the statistic:

$$\phi_x = \sigma_{xi}^2 / (\sum_i \sigma_{xi}) \quad \text{eq. 1}$$

This metric is constrained between zero and one, which represent asynchrony and synchrony, respectively. Additionally, we calculated an index of temporal stability of phytoplankton productivity as the ratio of mean biovolume to standard deviation across all sampling events (Lehman & Tilman, 2000; Isbell *et al.*, 2009). To analyze the amount of variability in temporal stability explained by fluctuations in synchrony, we used curve estimation regression analysis (SPSS Statistics18) using (a) all phytoplankton, (b) Cyanobacteria only, and (c) eukaryotic algae only. Then, to specifically address effects of synchrony in the Cyanobacteria on phytoplankton community stability, the same analysis was performed using synchrony calculated for Cyanobacteria alone and temporal stability in the entire phytoplankton community. The same methods were used to analyze the short term dataset.

Results

Biodiversity and community composition

We found variable timing, duration, and community composition of blooms in three lakes sampled in 2012. Lake Orient was the only lake with a sustained single genus bloom during the

month of July (*Microcystis* spp., Figure 1), while Keomah and Springbrook both had multi-genera bloom events and greater fluctuation in community structure (Figures 2 and 3).

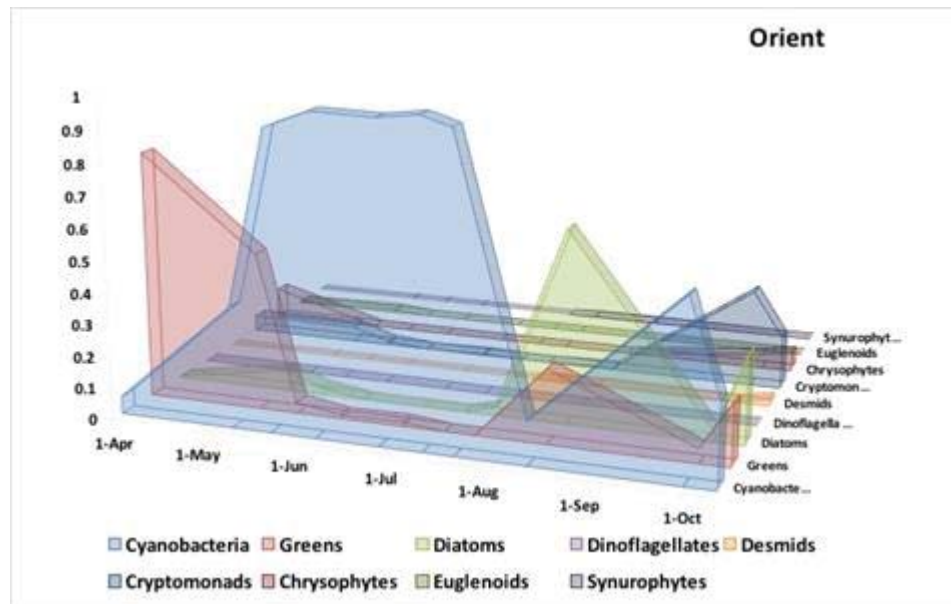


Figure 1. Community succession of phytoplankton orders in Lake Orient during the ice free season of 2012. Values on the y-axis represent relative abundance. Dominant Chlorophytes (Greens) in April were *Closteriopsis* sp.; Cyanobacteria between May and August included several species of *Microcystis* and *Aphanocapsa*; *M. wesenbergii* was dominant in mid-July.

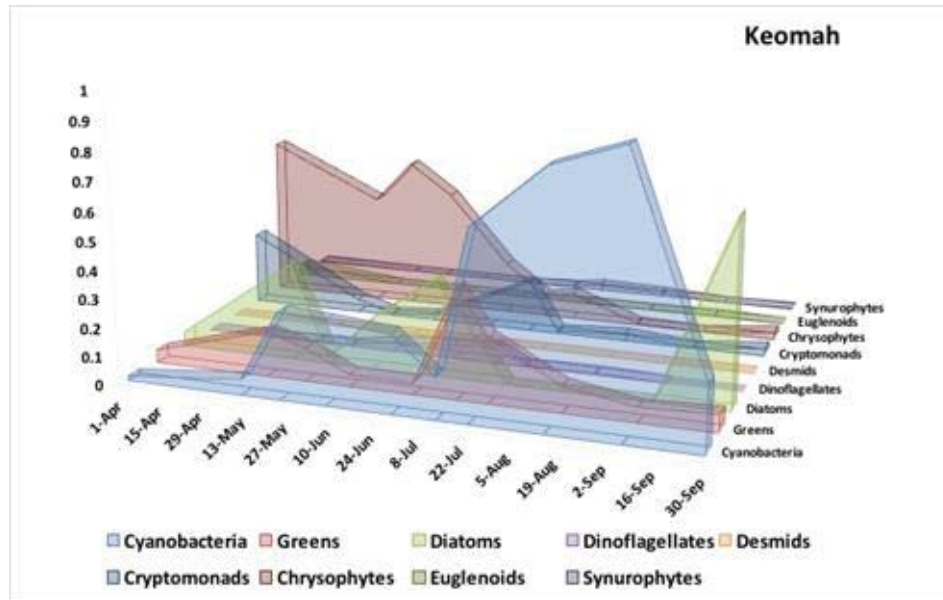


Figure 2. Community succession of phytoplankton orders in Lake Keomah during the ice free season of 2012. Values on the y-axis represent relative abundance.

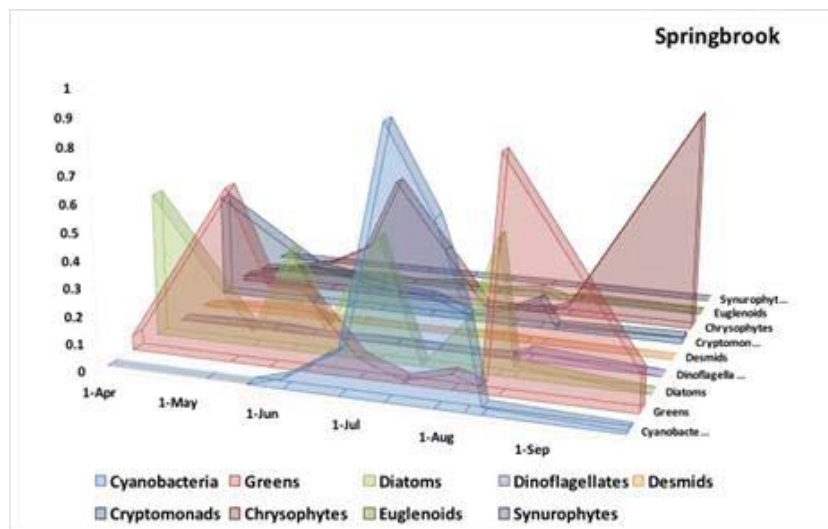


Figure 3. Community succession of phytoplankton orders in Springbrook Lake during the ice free season of 2012. Values on the y-axis represent relative abundance.

Patterns of genus level α -diversity (Shannon H) were also variable between lakes (Figure 4). While all three lakes were similarly diverse in early spring, Lake Keomah exhibited the highest seasonal biodiversity at the onset of the cyanobacteria bloom in mid-July, in contrast to Springbrook and Orient which had the lowest levels during this period. In all three lakes, the lowest levels of biodiversity correspond to cyanobacteria blooms, though a single genus bloom was only observed in hyper-eutrophic Lake Orient.

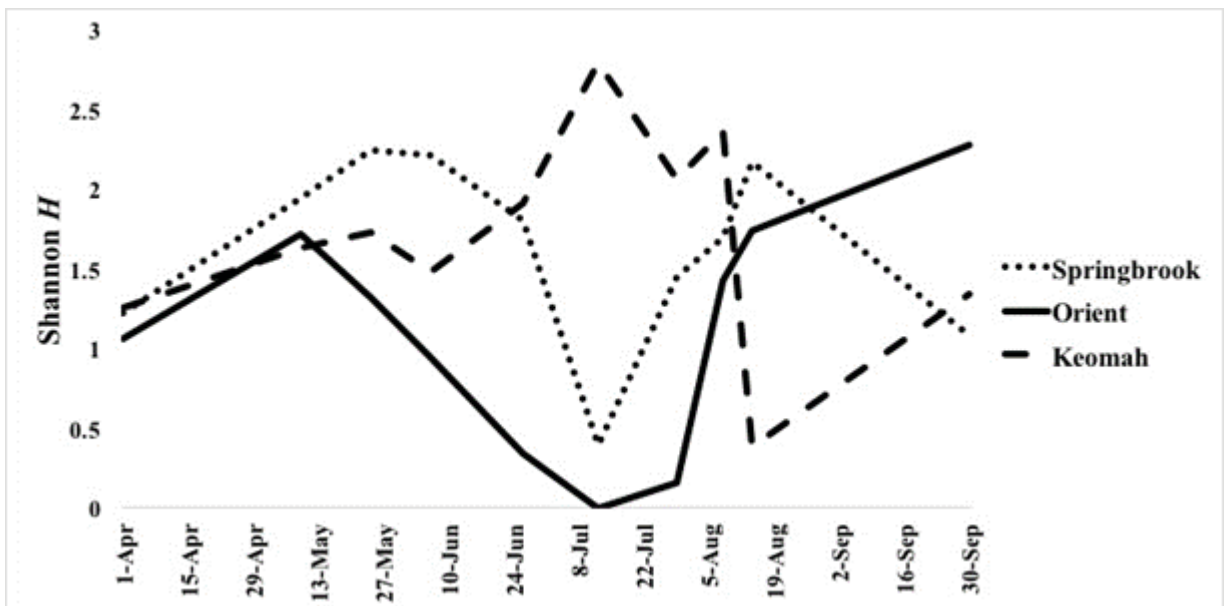


Figure 4. Time series of genus-level biodiversity in Springbrook, Orient, and Keomah. In all 3 cases, decreases in biodiversity correspond to cyanobacteria blooms.

Synchrony-stability relationships

In our long term dataset, significant negative exponential relationships were found between synchrony and temporal stability both at kingdom and phytoplankton community levels

(Table 1, Figure 5). The greatest amount of variability in temporal stability of productivity was explained by synchronous fluctuations in the eukaryotic algae ($R^2=0.78$, $F_{1,18}= 62.40$, $p<0.001$). Similar significant relationships were found when cyanobacteria were analyzed alone ($R^2=0.56$, $F_{1,18}= 22.93$, $p<0.001$) and when the phytoplankton community was analyzed as a whole ($R^2=0.56$, $F_{1,18}= 22.93$, $p<0.001$). It is evident from slopes of these relationships and visual inspection of curves that phytoplankton community dynamics follow patterns most similar to cyanobacteria constituents, while eukaryotic algae in general have lower temporal stability, synchrony, and variability around these trends. Additionally, phytoplankton community stability was strongly affected by synchronous fluctuations in the cyanobacteria ($R^2=0.55$, $F_{1,18}= 22.33$, $p<0.001$; Figure 2).

Table 1. Model parameters for regression analyses between synchrony (ϕ_x) and temporal stability (μ/σ) metrics of long term survey data.

x	y	Model	R^2	$F_{1,18}$	p
ϕ_{all}	μ/σ_{all}	$y=1.48e^{-1.64x}$	0.68	37.39	<0.001
ϕ_{cya}	μ/σ_{cya}	$y=1.51e^{-1.46x}$	0.46	22.93	<0.001
ϕ_{euk}	μ/σ_{euk}	$y=0.95e^{-1.75x}$	0.78	62.40	<0.001
ϕ_{cya}	μ/σ_{all}	$y=1.60e^{-1.45x}$	0.55	22.33	<0.001

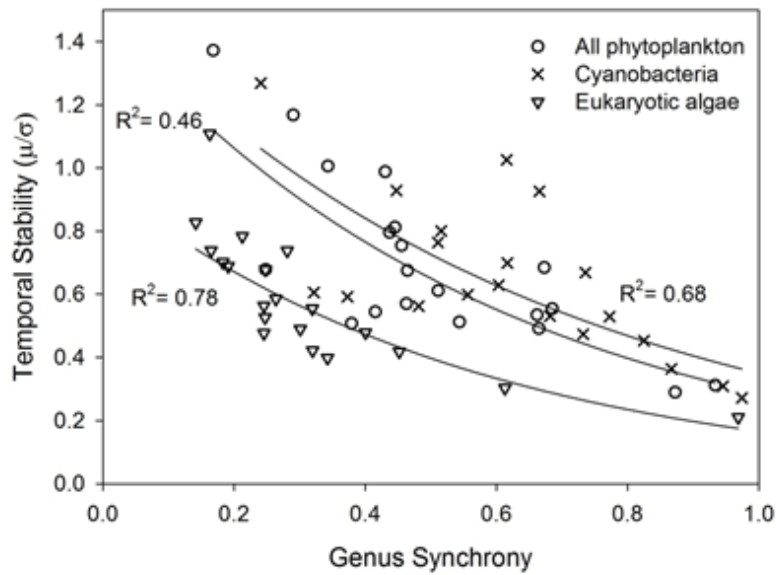


Figure 5. Negative exponential relationships between synchrony and temporal stability analyzed as the entire phytoplankton community (open circles), Cyanobacteria only (x), and eukaryotic algae only (inverted triangle) in 20 lakes, 2002-2010.

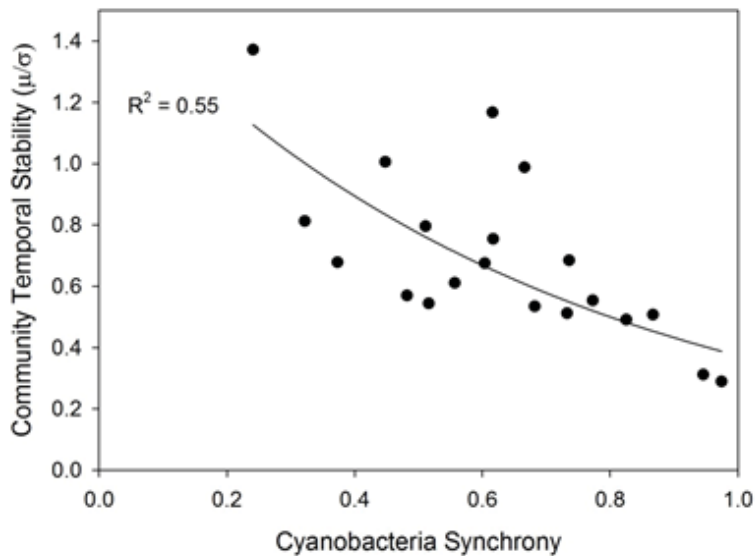


Figure 6. Negative exponential relationship between synchronous fluctuations in the Cyanobacteria and phytoplankton community stability in 20 lakes, 2002-2010.

When investigated at a higher frequency time scale in 2012, we again found similarity in relationships between cyanobacteria synchrony, cyanobacteria community stability, and phytoplankton community stability. At shorter time scales, however, community and cyanobacteria stability peaked at intermediate levels of genus synchrony in Lake Keomah, and was lowest at high levels of synchrony in Lake Orient. Eukaryotic algae showed the opposite trend, with the lowest stability at intermediate levels of synchrony in Lake Orient (Figure 8).

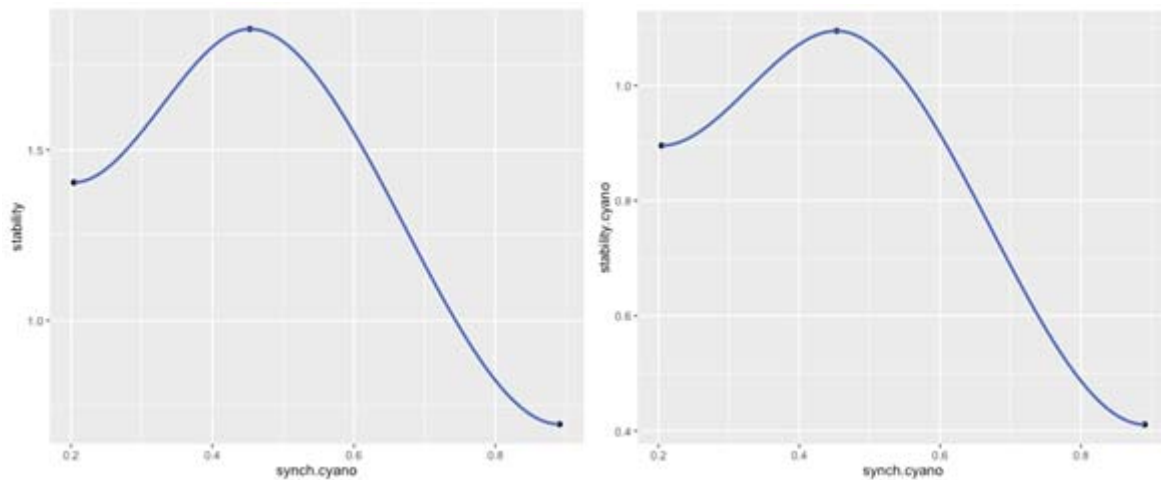


Figure 7. Relationships between community stability and cyanobacteria synchrony (left) and cyanobacteria stability and cyanobacteria synchrony (right) in Lakes Orient, Keomah, and Springbrook in 2012.

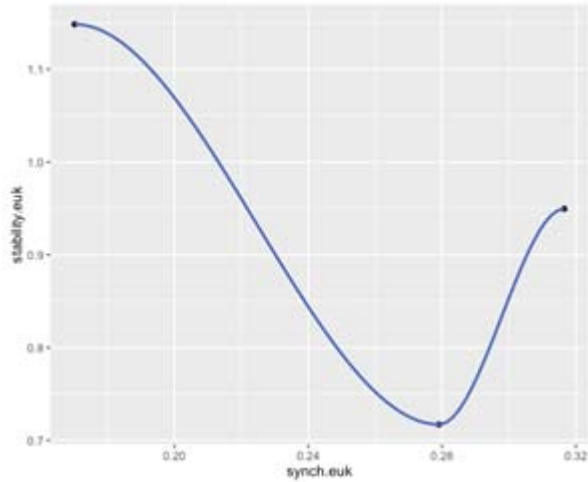


Figure 8. Relationship between eukaryotic community stability and synchrony in Lakes Orient, Keomah, and Springbrook in 2012.

Discussion

Our results indicate that through cyclical bloom dynamics and periodic dominance, cyanobacteria communities can act to lessen the temporal stability of productivity in eutrophic lake phytoplankton communities. It is significant to note that these patterns do not occur via a linear decline in stability, suggesting a rapid and compounded community response to synchronous fluctuations. In addition, these effects resulted in a steeper decline of stability in the entire community and cyanobacteria than in the eukaryotic community alone. Similarities in relationships between synchrony and whole community stability with cyanobacteria stability demonstrate that fluctuations in dominant cyanobacterial communities are driving phytoplankton community dynamics. These results have serious implications for lake ecosystem function, particularly in terms of carbon cycling and gas flux.

Results of this study provide support for theoretical predictions that synchronous fluctuation will increase with large fluctuations in community biomass and cyclical or chaotic processes, and that biodiversity can regulate temporal variability in communities (Loreau & de Mazancourt, 2008, 2013). The relationship between biodiversity and temporal variability of communities has been investigated and debated for decades (Cottingham and others 2001, as referenced therein). Early work by MacArthur (1955) and Elton (1958) established a relationship between stability and complexity (richness) of communities, which was later supported empirically by McNaughton (1978) among others, though these relationships were contested by May, whose theoretical work demonstrated that complex communities are the least stable (May, 1972a, 1972b; May & MacArthur, 1972).

Over long temporal scales, our work suggests that cyclic fluctuations drive instability in primary producer communities. On shorter time scales, perhaps more relevant to turnover times of microbial communities, we found variable responses of community stability to fluctuations in prokaryotic cyanobacteria versus eukaryotic algae. Intermediate fluctuations in cyanobacteria populations were associated with community stability, while large fluctuations resulted in instability. We found the opposite result for eukaryotic communities, which were the least stable at intermediate levels of community synchrony. Results for cyanobacteria communities are consistent with the intermediate disturbance hypothesis described by Connell (1978) and revisited in the context of phytoplankton communities by Sommer (1993), where the greatest diversity would be expected at intermediate levels of disturbance intensity or frequency. In this context, the disturbance would be synchronous fluctuations in cyanobacteria communities, though this is also testable in terms of environmental fluctuation.

Fluctuations in aquatic primary producer communities drive ecosystem scale processes, making it critical to understand drivers of community stability and instability across trophic gradients. Global increases in cyanobacteria blooms represent large scale loss of aquatic biodiversity which has fundamentally altered aquatic ecosystem function, and the interaction of lakes with global carbon cycles (Balmer and Downing 2011; Heathcote and Downing 2011; Jones and others 2016; Morales-Williams and others 2016 a,b in review, Chapters 2 and 3). Our results demonstrate that fluctuations in cyanobacteria populations can result in primary producer community instability, though this response is variable over long and short time scales. These community fluctuations represent a disturbance process which may destabilize biogeochemical processes at the ecosystem scale.

CHAPTER VI

SUMMARY AND CONCLUSIONS

My dissertation research demonstrated that eutrophication has fundamentally altered lake ecosystem functioning at multiple ecological and temporal scales. The five major findings of my research are, 1. Cyanobacteria biomass can be maintained via a carbon concentrating mechanism and HCO_3^- uptake when CO_2 is depleted, 2. Eutrophic lakes can be net CO_2 sinks on an annual basis and can remain under-saturated with CO_2 both day and night relative to the atmosphere for more than a month at a time, 3. Eutrophic lakes that are net sources of CO_2 can be large sources, with rates comparable to boreal ecosystems., 4. Nitrite photodegradation, hydroxyl radical production, and DOM quality contribute to elevated CO_2 concentrations., and 5. Synchronous fluctuations in cyanobacteria populations destabilize phytoplankton communities at long and short temporal scales. Taken together, these findings demonstrate that eutrophication has altered both biogeochemical carbon cycling and the maintenance of communities in these systems.

In Chapter 2, I demonstrated that phytoplankton communities shift rapidly to an alternative carbon assimilation mechanism when surface water CO_2 drops below atmospheric equilibrium. When examined together with patterns of biodiversity in lakes Springbrook, Orient, and Keomah from Chapter 4, biodiversity decreases with % cyanobacteria abundance, and increases with photosynthetic fractionation (Figure 1), further demonstrating that the carbon concentrating mechanism maintains cyanobacteria dominance when CO_2 is depleted in surface waters.

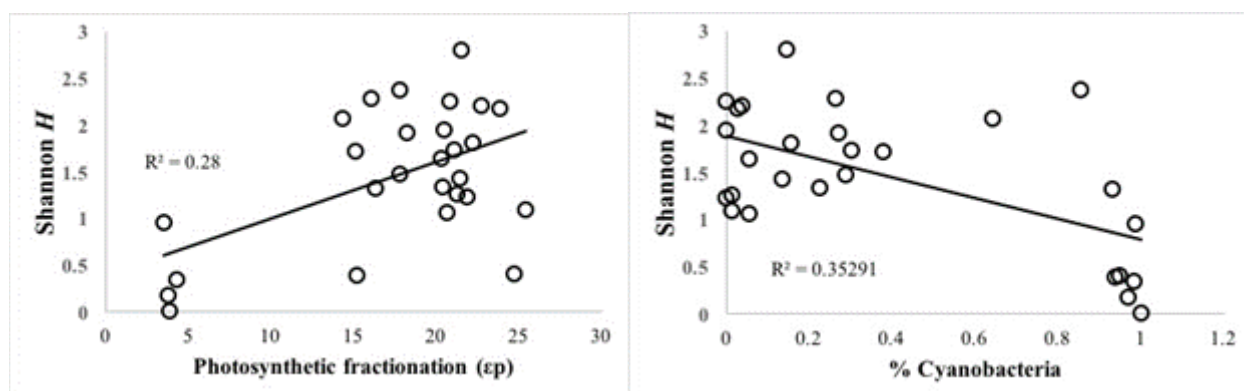


Figure 1. Linear relationships between Shannon diversity and % cyanobacteria dominance (left) and photosynthetic fractionation (right), demonstrating decreasing in biodiversity with cyanobacteria dominance and HCO_3^- utilization.

Findings in Chapter 3 revealed extended periods of CO_2 influx yielding 1/3 of lakes studied net CO_2 sinks during the ice free season. In addition to this, I show that the dissolved organic matter pool is autochthonous in origin, and that there is no stable isotopic evidence of degradation of terrestrial organic matter. These findings are important because our understanding of carbon cycling in lakes is largely based work from north temperate and boreal ecosystems dominated by inputs of terrestrial organic matter (Cole *et al.*, 1994, 2011; Pace *et al.*, 2004; Maberly *et al.*, 2013; Wilkinson *et al.*, 2016). While several notable studies have emerged from the agricultural Midwestern US (Balmer & Downing, 2011; Heathcote & Downing, 2011; Pacheco *et al.*, 2014; Jones *et al.*, 2016), more work needs to be done globally to understand the role of these important and active systems on a global scale.

An interesting observation that emerged from results of Chapter 3 was that while several lakes were net CO_2 sinks, others were large sources of CO_2 to the atmosphere, with rates comparable to boreal ecosystems. The question that emerged from this observation was: how can highly productive lakes with minimal contributions of terrestrial organic matter generate such large effluxes of inorganic carbon? Why does primary production in these lakes not draw

down CO₂? The commonality among these lakes was high concentrations of nitrate, leading to the hypothesis that nitrate in surface waters is rapidly photodegraded yielding hydroxyl radicals that mineralize organic carbon (Schwarzenbach *et al.*, 2003). While I found a significant relationship between nitrate and CO₂ concentration in 2012, experimental results in 2013 did not demonstrate a direct relationship. Instead, accumulation of nitrite, not nitrate, drove reactive oxygen production, and the quality of the organic matter pool was strongly related to ambient CO₂.

In Chapter 4 I explored ecological mechanisms that act to destabilize phytoplankton communities in eutrophic lakes. Specifically, I found that phytoplankton community stability is strongly related to synchronous fluctuations of bloom-forming cyanobacteria, though these relationships differ on short and long time scales. When analyzed annually over a decade, stability decreases exponentially with increased synchronous fluctuations in the cyanobacteria. When analyzed bi-weekly over one ice-free season, phytoplankton community stability was greatest at intermediate levels of synchrony. The long term findings are consistent with similar work in terrestrial ecosystems demonstrating that species synchrony destabilizes temporal stability of productivity in grasslands (Isbell *et al.*, 2009). Analysis on a short term scale revealed an intermediate optimum in genus-level cyanobacteria synchrony at which phytoplankton communities are most stable. Though these results would be better supported by including more lakes in the analysis, this pattern suggests that eutrophic lake phytoplankton communities are most stable at intermediate levels of disturbance (e.g., Connell, 1978).

In summary, my dissertation research demonstrates that human impacts to aquatic ecosystems have resulted in fundamental changes to their ecology and biogeochemistry. These systems are ecological hotspots of biogeochemical processing and transformation that will

continue to increase as human populations expand. Developing a mechanistic understanding of processes that drive community assembly and biogeochemical processing in these lakes is critical to assessing the role of inland waters in global cycles. Too often, eutrophic lakes are excluded from models because their processes and rates do not fit within our paradigm of how lakes should behave. More studies are needed globally to assess the extent eutrophic lakes worldwide, and better understand their contribution to carbon and nutrient flux, export, and storage.

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APPENDIX

CHAPTER 3 SUPPORTING INFORMATION

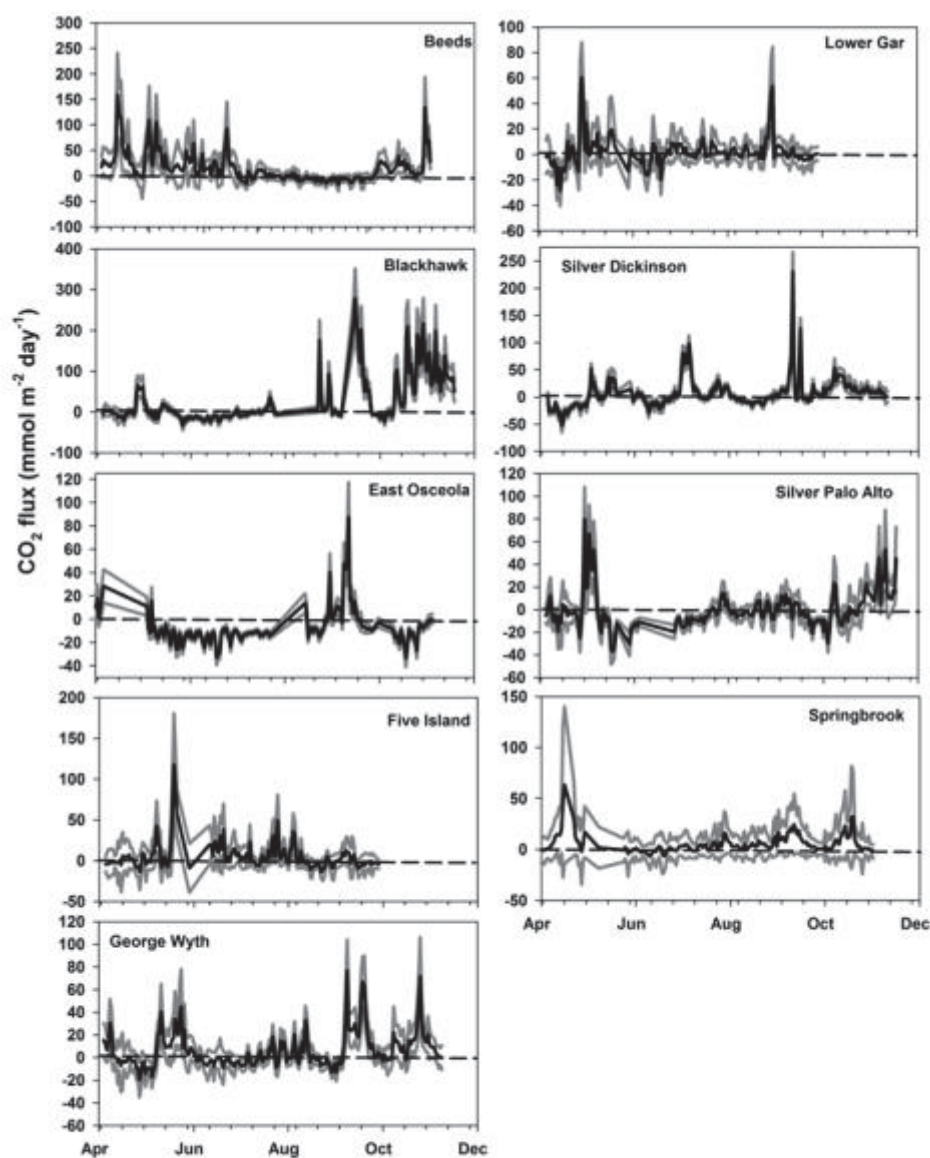


Figure S1. Net flux ($\text{mmol C m}^{-2} \text{d}^{-1}$) during the ice free season for 9 lakes in this study. Net flux was calculated as the sum of average daily flux measured continuously over the study period. Black lines are modeled flux; grey lines are 95% confidence intervals.

Table S1. Mean \pm standard deviation of limnological variables and optical indices measured in this study across sampling events. FI is the fluorescence index, HIX_{ohmo} is the humification index. Epilimnion temperature and specific conductivity are mean values of epilimnetic vertical profile.

Lake	Alkalinity (mg CaCO ₃ L ⁻¹)	DO (mg L ⁻¹)	Depth (m)	Thermocline Depth (m)	FI	$\beta:\alpha$	HIX_{ohmo}	Specific Conductivity (mS cm ⁻¹)	Epilimnion Temp (°C)
Arrowhead	189.7 \pm 8.4	9.5 \pm 1.0	5.05 \pm 0.25	1.74 \pm 1.85	1.62 \pm 0.03	0.82 \pm 0.05	0.72 \pm 0.06	0.5 \pm 0.0	24.0 \pm 5.3
Badger	169.8 \pm 33.4	11.3 \pm 2.6	4.54 \pm 0.17	1.45 \pm 1.29	1.64 \pm 0.05	0.74 \pm 0.05	0.87 \pm 0.02	0.5 \pm 0.1	22.0 \pm 5.4
Beeds	189.8 \pm 37.0	13.0 \pm 2.6	6.64 \pm 0.27	2.34 \pm 1.20	1.59 \pm 0.03	0.73 \pm 0.03	0.87 \pm 0.04	0.5 \pm 0.1	23.3 \pm 4.6
Blackhawk	187.3 \pm 12.1	9.6 \pm 2.4	2.01 \pm 0.26	0.00 \pm 0.00	1.56 \pm 0.03	0.79 \pm 0.02	0.81 \pm 0.09	0.5 \pm 0.0	22.3 \pm 6.1
Center	160.5 \pm 4.2	13.1 \pm 2.7	4.30 \pm 0.14	0.00 \pm 0.00	1.69 \pm 0.05	0.83 \pm 0.04	0.79 \pm 0.02	0.6 \pm 0.0	15.9 \pm 4.3
E. Osceola	111.7 \pm 27.0	12.0 \pm 4.1	3.95 \pm 0.17	1.84 \pm 0.76	1.61 \pm 0.06	0.74 \pm 0.03	0.86 \pm 0.03	0.3 \pm 0.1	22.8 \pm 3.4
Five Island	163.5 \pm 8.1	8.3 \pm 3.4	5.65 \pm 0.42	0.31 \pm 0.84	1.57 \pm 0.08	0.74 \pm 0.02	0.87 \pm 0.04	0.4 \pm 0.0	22.8 \pm 4.8
George Wyth	142.0 \pm 28.0	9.5 \pm 2.2	3.58 \pm 0.11	0.94 \pm 1.23	1.59 \pm 0.02	0.78 \pm 0.04	0.80 \pm 0.02	0.6 \pm 0.0	23.3 \pm 4.4
Keomah	116.3 \pm 16.6	11.7 \pm 2.9	5.13 \pm 0.68	1.47 \pm 1.45	1.61 \pm 0.03	0.78 \pm 0.03	0.82 \pm 0.02	0.3 \pm 0.1	24.5 \pm 4.5
Orient	94.4 \pm 24.7	12.7 \pm 3.2	2.61 \pm 0.15	0.56 \pm 0.85	1.62 \pm 0.05	0.80 \pm 0.05	0.74 \pm 0.09	0.3 \pm 0.0	23.1 \pm 5.6
Lower Gar	186.3 \pm 13.6	11.4 \pm 1.9	1.43 \pm 0.16	0.00 \pm 0.00	1.56 \pm 0.04	0.75 \pm 0.02	0.84 \pm 0.01	0.4 \pm 0.0	22.0 \pm 4.9
Rock Creek	148.5 \pm 7.5	9.9 \pm 2.2	4.48 \pm 0.13	0.98 \pm 1.24	1.61 \pm 0.03	0.76 \pm 0.03	0.85 \pm 0.02	0.4 \pm 0.0	24.1 \pm 4.2
Silver-D	170.4 \pm 13.5	9.6 \pm 1.7	2.37 \pm 0.14	0.00 \pm 0.00	1.60 \pm 0.06	0.75 \pm 0.03	0.88 \pm 0.01	0.6 \pm 0.0	21.8 \pm 4.8
Silver-PA	174.9 \pm 28.0	9.4 \pm 2.9	1.54 \pm 0.21	0.00 \pm 0.00	1.59 \pm 0.08	0.77 \pm 0.03	0.84 \pm 0.06	0.4 \pm 0.0	20.3 \pm 6.9
Springbrook	183.5 \pm 22.0	11.2 \pm 2.8	5.43 \pm 0.61	2.55 \pm 0.83	1.69 \pm 0.04	0.77 \pm 0.05	0.80 \pm 0.05	0.4 \pm 0.0	23.8 \pm 4.0